West Hertfordshire Hospitals NHS
NHS Trust

FNA, ROSE and ancillary tests

Principles and Practice

Dr Tony Maddox
Consultant cyto/histopathologist

ROSE – what does it mean?

Rapid OnSite Evaluation, but…

- To what end?
- Using what methods?
- Performed by whom?
- And, in the literature, reported by whom?

FNA - postulates

Cellular material obtained by FNA has potentially critical diagnostic value

Value should be maximised taking account of FNA site and treatment options

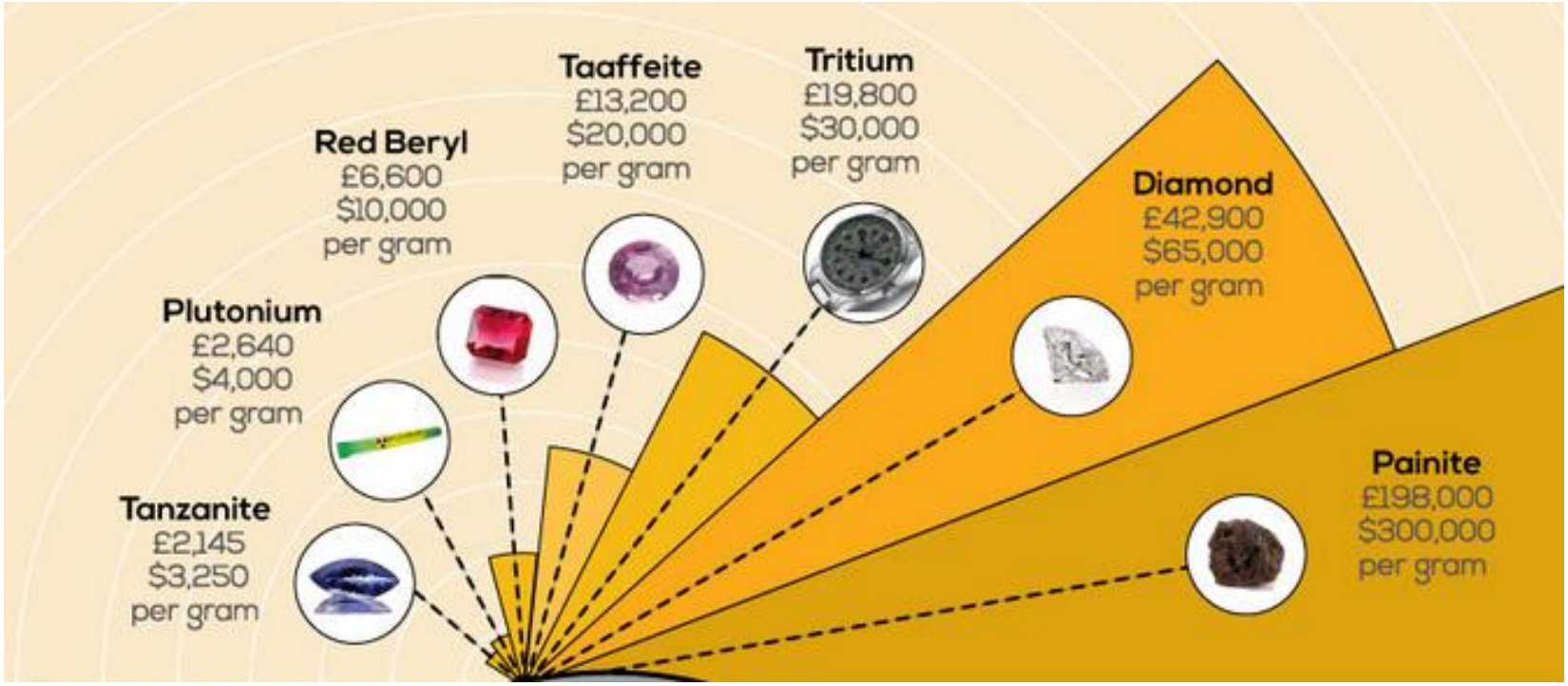
EBUS tissue – (monetary) value

- Single FNA weighs about:
  - 10mg
- NHS tariff for EBUS is:
  - £1276
- Assume 5 passes (50mg), EBUS tissue is worth:

–£25,520/gram

Kreula et al. Br J Surg 1989;**76**: 1270-1272

EBUS tissue - £25,520/gram



Potential benefits of ROSE

Diagnostic

- Adequacy
- Diagnostic yield
  - % of cases with an actual diagnosis
  - May be specified for a particular diagnosis
  - Sensitivity, specificity, PPV, NPV
- Accuracy
  - Comparison with “gold standard”

Potential benefits of ROSE

Process

- Number of passes
- Number of sites
- Procedure time/resources
- Cost
- Repeat procedures

Potential benefits of ROSE

Ancillary tests

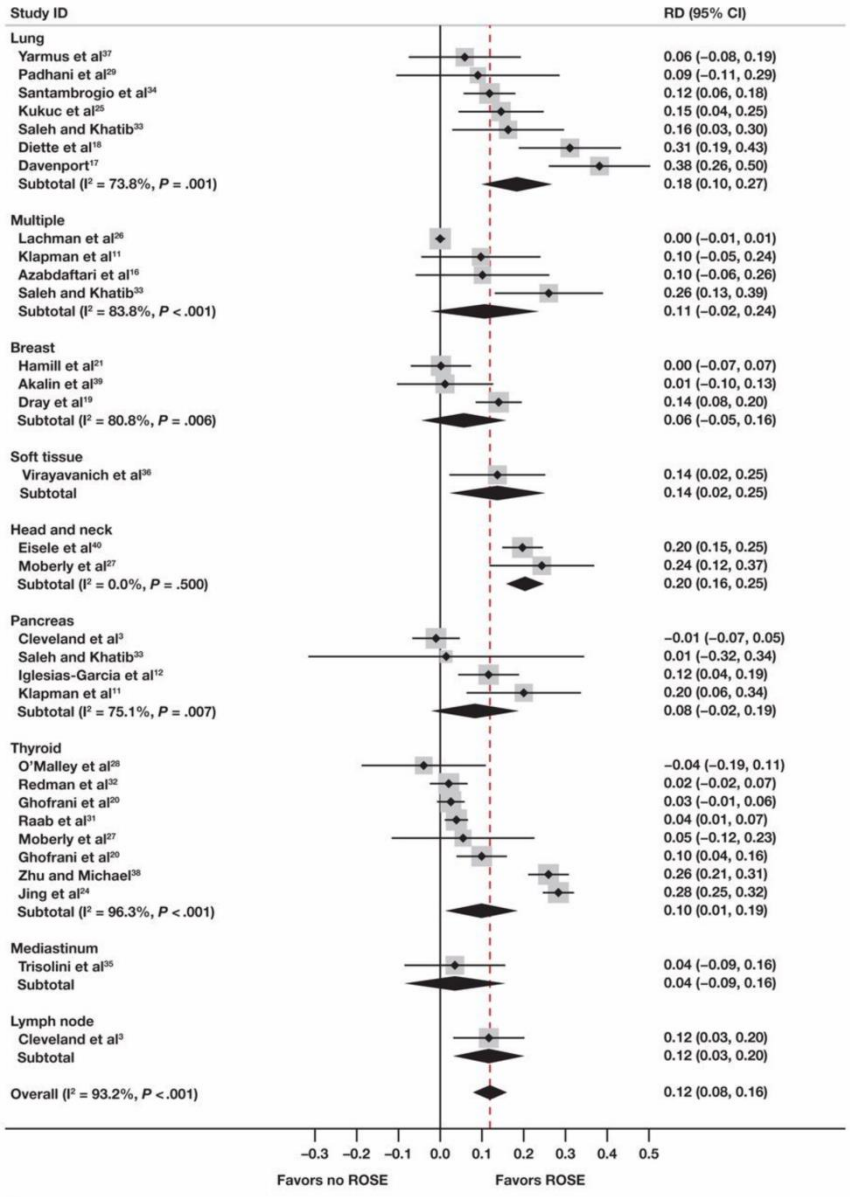
- Immunocytochemistry
  - Diagnostic, predictive
- Molecular (mutations, translocations)
  - Predictive, prognostic
- Flow cytometry
  - Diagnostic
- Microbiological

Main sites covered today

- Mediastinum (EBUS/EUS)
- Pancreas (EUS)
- Head and neck

Mediastinum

adequacy



The Influence of Rapid Onsite Evaluation on the Adequacy Rate of Fine-Needle Aspiration Cytology. A Systematic Review and Meta-Analysis.

Schmidt RL et al

Am J Clin Pathol. 2015;139(3):300-308.
doi:10.1309/AJCPEGZMJKC42VUP

Meta-analysis of 25, 2-cohort, studies with and without ROSE, a total of 12,407 cases

Forest plot shows change in adequacy rate when ROSE used. Analysis is not adjusted for initial adequacy.

Table 2—*Results of the Outcome Measures*

Measure	TBNA (n = 85)	TBNA + ROSE (n = 83)	*P* Value
Diagnostic yield,[a] No. (%)	64 (75.3)	65 (78.3)	.64
Adequate samples,[b] No. (%)	109 (86.5)	80 (78.4)	.10
Number of biopsy sites,[a] median (IQR)	2 (1-2)	1 (1-2)	.0005[c]
Complication rate of bronchoscopy,[a] No. (%)	17 (20)	5 (6)	.011[c]

Rapid On-site Evaluation of Transbronchial Aspirates in the Diagnosis of Hilar and Mediastinal Adenopathy

Trisolini et al

CHEST 2011; 139(2):395–401

168 patients randomised to conventional TBNA with and without ROSE

Adequacy – “a preponderance of lymphocytes”

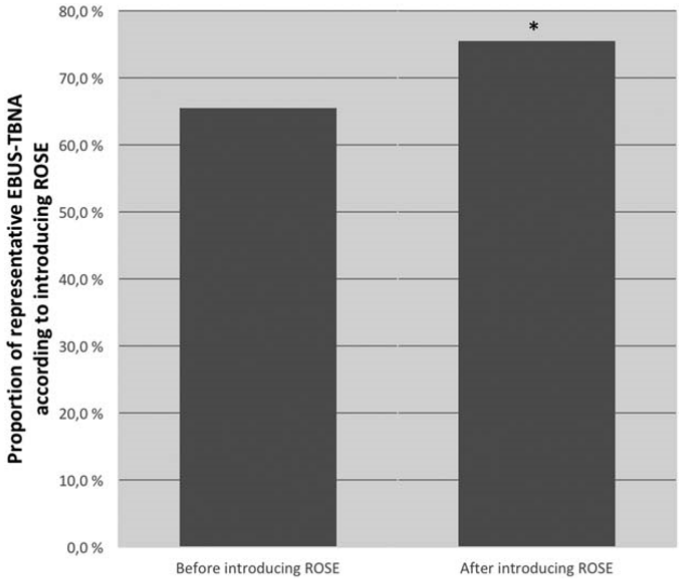


Figure 3. Proportion of representative EBUS-TBNA before and after introducing rapid on-site cytological evaluation (ROSE) by experienced cytotechnologists. Data are presented as % of all EBUS-TBNA. *: $P = 0{,}003$ compare to before ROSE.

"Diagnostic performance"

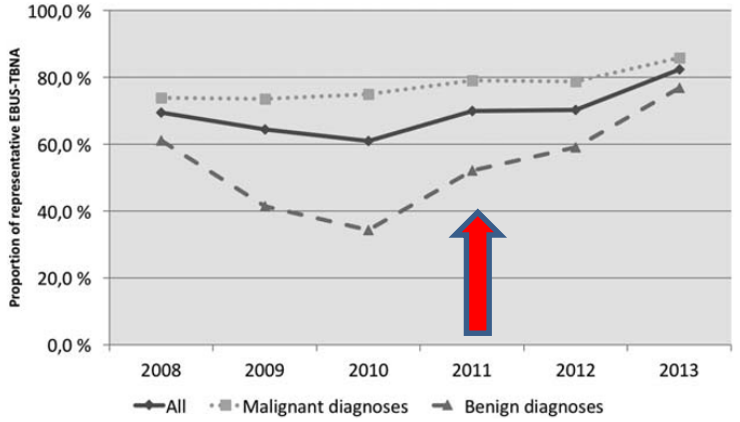


Figure 1. Proportion of representative EBUS-TBNA according to a final malignant or benign diagnosis. Data are presented as % of all EBUS-TBNA.

Learning endobronchial ultrasound transbronchial needle aspiration – a 6-year experience at a single institution

Sveinung Sørhaug et al
Clin Respir J 2018; 12: 40–47

711 EBUS (855 sites), 299 (368) before ROSE, 412 (487) after ROSE

Adequacy: >40 lymphocytes per x40f
ROSE provided by cytotechnologists

Adequacy in the mediastinum

- Alsharif (Minnesota - 2008)
  - 40 lymphocytes/x40f in most cellular area
  - OR pigmented macrophages
  - OR diagnostic material
- Nayak (New York - 2010)
  - (5 x 100 lymphocytes/x10f AND <2 bronchial cell groups/x10f)
  - OR germinal centre fragments
  - OR diagnostic material

Adequacy in the mediastinum

- x10f has 16 times greater area than x40f
- 40 lymphocytes/x40f = 640 lymphocytes/x10f

- 5 x 100 lymphocytes/x10f = 500 lymphocytes

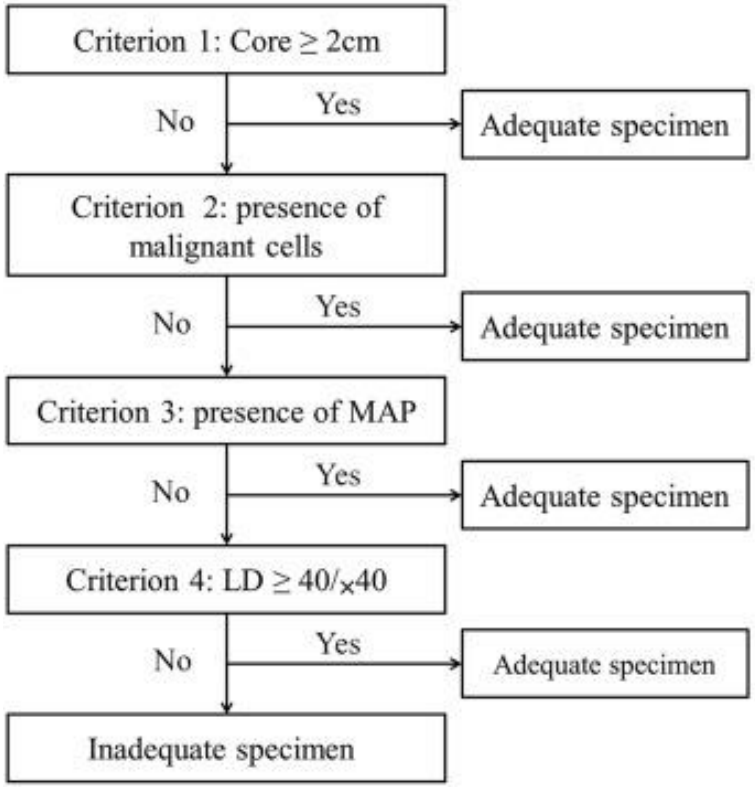
Adequacy in the mediastinum

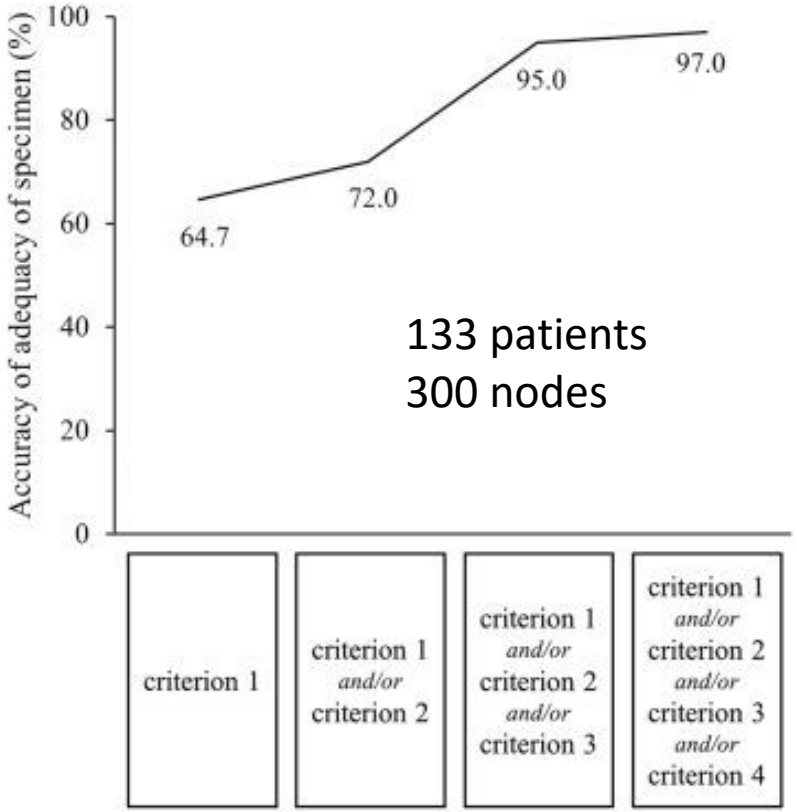
	New York		
Minnesota	Adequate	Unsatisfactory	Total
Adequate, No. (%)	100 (85)	2 (2)	102 (86)
Unsatisfactory, No. (%)	0 (0)	16 (14)	16 (14)
Total, No. (%)	100 (85)	18 (15)	118 (100)
Simple κ		McNemar's Test	
κ	0.931	χ^2	2.000
Standard error	0.048	*df*	1
95% confidence limits	0.837-1.000	*P*	.16

Abbreviation: UAMS, University of Arkansas for Medical Sciences.

Jeffus et al. Rapid On-Site Evaluation of EBUS-TBNA Specimens of Lymph Nodes: Comparative Analysis and Recommendations for Standardization. *Cancer Cytopathol.* 2015;123:362-72

Adequacy for physicians





Choi et al, *The Annals of Thoracic Surgery* 2016 101(2), 444-450

Adequacy in the mediastinum

- Does ROSE help?
  - Evidence suggests:
  - yes if the adequacy rate is low (<75%)
  - no if the adequacy rate is ok (>75%)
- Nevertheless, need reproducible criteria
  - We use 40 lymphocytes/x40f or pigmented macrophages or diagnostic material.

Mediastinum

Diagnostic yield and accuracy

Table 2—*Results of the Outcome Measures*

Measure	TBNA (n = 85)	TBNA + ROSE (n = 83)	*P* Value
Diagnostic yield,[a] No. (%)	64 (75.3)	65 (78.3)	.64
Adequate samples,[b] No. (%)	109 (86.5)	80 (78.4)	.10
Number of biopsy sites,[a] median (IQR)	2 (1-2)	1 (1-2)	.0005[c]
Complication rate of bronchoscopy,[a] No. (%)	17 (20)	5 (6)	.011[c]

Rapid On-site Evaluation of Transbronchial Aspirates in the Diagnosis of Hilar and Mediastinal Adenopathy

Trisolini et al

CHEST 2011; 139(2):395–401

168 patients randomised to conventional TBNA with and without ROSE

Overall diagnostic yield – ROSE 85%, non-ROSE 75%, p=0.23

Table 4. Diagnostic value of EBUS-TBNA for lung cancer

	ROSE (n = 55)	Non-ROSE (n = 53)
Sensitivity	88	86
Specificity	100	100
Positive predictive value	100	100
Negative predictive value	40	63
Accuracy[a]	89	89

Data are presented as %. [a] $p = 0.95$ using χ^2 test.

Rapid On-Site Cytologic Evaluation during Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration for Diagnosing Lung Cancer: A Randomized Study

Oki et al

Respiration 2013;85:486–492

108 patients randomised to EBUS-TBNA with and without ROSE

Diagnostic yield and diagnostic accuracy for lung cancer secondary endpoints

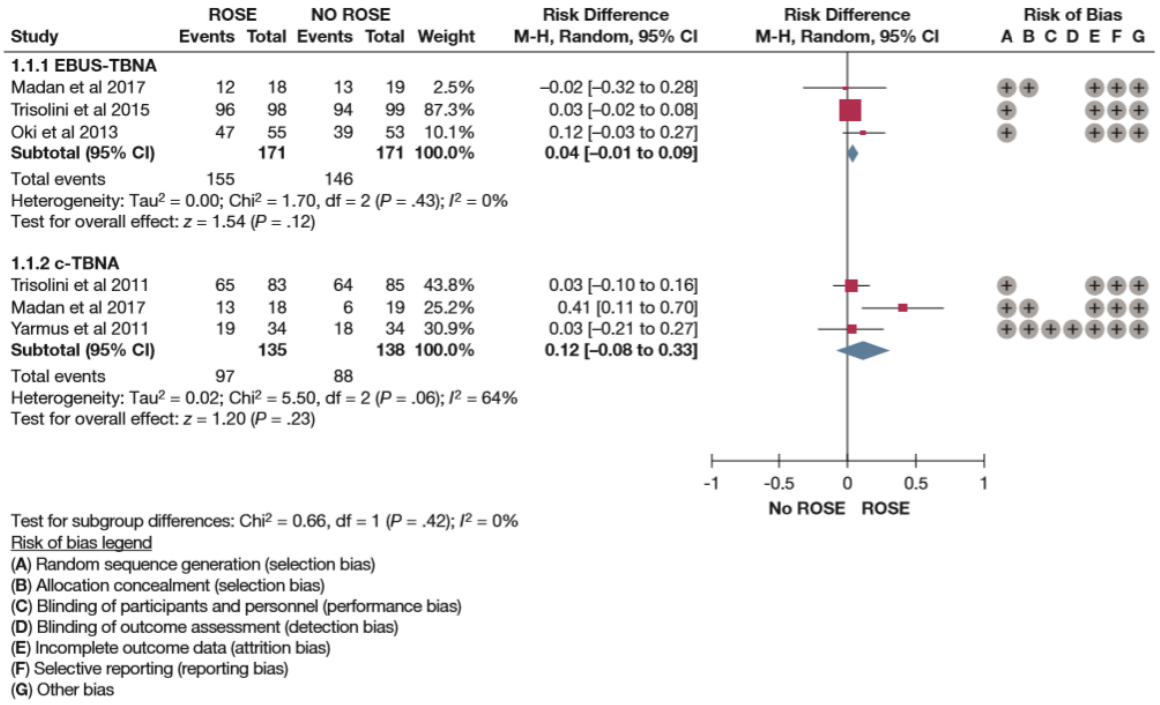


Figure 2 – *Forest plot of the risk difference comparing the diagnostic yield of EBUS-TBNA and c-TBNA with or without ROSE. The risk difference of individual studies is represented by a square through which runs a horizontal line (95% CI). The diamond with horizontal lines represents the pooled risk difference with 95% CI. Also depicted is the risk of bias of the individual studies. c-TBNA = conventional TBNA; M-H = Maentel-Hanszel test. See Figure 1 legend for expansion of other abbreviations.*

Impact of Rapid On-Site Cytological Evaluation (ROSE) on the Diagnostic Yield of Transbronchial Needle Aspiration During Mediastinal Lymph Node Sampling: Systematic Review and Meta-Analysis.

Sehgal et al
CHEST 2018; 153(4):929-938

5 studies – 618 subjects – good quality.
No effect of ROSE on diagnostic yield in EBUS or c-TBNA

Diagnostic yield - mediastinum

- Does ROSE help?
  - Evidence suggests:
  - No (even in blind TBNA)

West Hertfordshire Hospitals NHS
NHS Trust

Mediastinum

Process

Table 2—*Results of the Outcome Measures*

Measure	TBNA (n = 85)	TBNA + ROSE (n = 83)	*P* Value
Diagnostic yield,[a] No. (%)	64 (75.3)	65 (78.3)	.64
Adequate samples,[b] No. (%)	109 (86.5)	80 (78.4)	.10
Number of biopsy sites,[a] median (IQR)	2 (1-2)	1 (1-2)	.0005[c]
Complication rate of bronchoscopy,[a] No. (%)	17 (20)	5 (6)	.011[c]

Rapid On-site Evaluation of Transbronchial Aspirates in the Diagnosis of Hilar and Mediastinal Adenopathy

Trisolini et al

CHEST 2011; 139(2):395–401

168 patients randomised to conventional TBNA with and without ROSE

Significant reduction in targeted sites

TABLE 2] Procedural Details (per Patient Analysis)

Procedural Detail	Overall Population (N = 197)		
	ROSE (98)	EBUS (99)	*P* Value
Duration, mean (SD),[a] min	17.8 (8.34)	17.9 (5.61)	.871
No. sampled sites[b]			.005[c]
1	76 (55.9)	60 (44.1)	
2	19 (33.3)	38 (66.7)	
3	3 (75)	1 (25)	

Randomized Trial of Endobronchial UltrasoundGuided Transbronchial Needle Aspiration With and Without Rapid On-site Evaluation for Lung Cancer Genotyping

Trisolini et al

CHEST 2015; 148(6):1430-1437

197 patients randomised to EBUS TBNA with and without ROSE

Significant reduction in targeted sites

TABLE 2. Improved Health Care Resource Utilization With Rapid On-Site Evaluation (ROSE) Endobronchial Ultrasound Fine-Needle Aspiration Biopsy: Analysis of Biopsy Sites

Number of Biopsy Sites	Non-ROSE (340 Patients)	ROSE (340 Patients)	Difference (Absolute #)	Difference (Proportional)	Significance (*P* Value[a])
1 Biopsy Site	122 (35.88%)	231 (67.94%)	109 (47.1%)	0.3206	<.0001
2 or More Biopsy Sites	218 (64.12%)	110 (32.35%)	−108 (49.5%)	−0.3176	<.0001
3 or More Biopsy Sites	113 (33.23%)	22 (6.47%)	−91 (80.5%)	−0.2676	<.0001
4 or More Biopsy Sites	34 (10.0%)	1 (0.29%)	−33 (97.0%)	−0.0971	na[b]
Total Biopsy Sites	709	474	−235 (33.1%)		

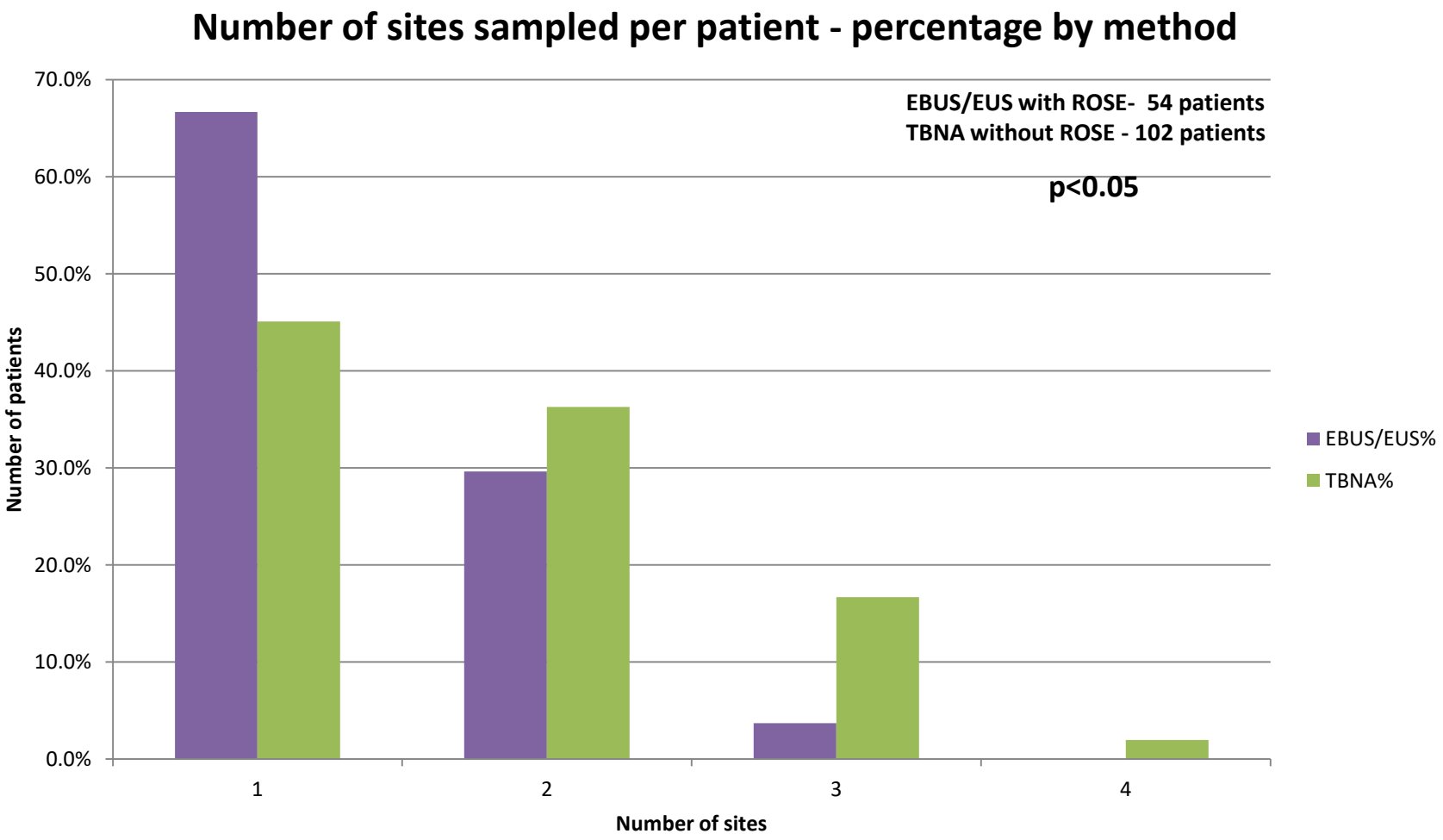
Improved Laboratory Resource Utilization and Patient Care With the Use of Rapid On-Site Evaluation for Endobronchial Ultrasound Fine-Needle Aspiration Biopsy
Collins BT et al
Cancer (Cancer Cytopathol) 2013;121:544-51.

Matched case-control cohorts of TBNA with and without ROSE (340 each).

Mean sites/patient 2.085 > 1.394
33% reduction in sites biopsied

Mean slides/site 8.42 > 8.824
Ie no significant change

West Herts


Number of sites sampled per patient - percentage by method
EBUS/EUS with ROSE- 54 patients
TBNA without ROSE - 102 patients
p<0.05
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
Number of patients
1
2
3
4
Number of sites
EBUS/EUS%
TBNA%

Do any studies show reduction in passes/site?

Table 2. Procedural details

Variables	ROSE (n = 55)	Non-ROSE (n = 53)	p value
Mean puncture number for main target lesion	2.2±0.9 (1–6)	3.1±0.4 (3–5)	<0.001
Additional procedures	6	30	<0.001
EBUS-TBNA for other lesions	2	26	
TBB for peripheral lesions	4	3	
EBUS-TBNA for other lesions and TBB for peripheral lesions	0	1	

Rapid On-Site Cytologic Evaluation during Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration for Diagnosing Lung Cancer: A Randomized Study

Oki et al

Respiration 2013;85:486–492

108 patients randomised to EBUS-TBNA with and without ROSE

No of needle passes was a secondary endpoint

TABLE 4. Improved Health Care Resource Utilization With Rapid On-Site Evaluation (ROSE) Endobronchial Ultrasound (EBUS) Fine-Needle Aspiration Biopsy: Analysis of Health Care Utilization and Service Impact

Service Impact Category	Non-ROSE	ROSE	Difference (Absolute #)	Time Effect		
				Minutes	Hours	Days
Cytotechnologist work effort						
Total number of slides	5973 slides	4183 slides	−1790			
Time calculation[a]				8950 minutes	149.2 hours	18.6 working days[b]
Cytopathologist work effort						
Total number of slides	5973 slides	4183 slides	−1790			
Time calculation[c]				5370 minutes	89.5 hours	11.19 working days[b]
EBUS procedural time						
Biopsy sites	709	474	−235			
Time calculation[d]				3525 minutes	58.75 hours	7.3 working days[b]

[a] Cytotechnologist time calculation: 5.0 minutes per slide (5.0 minutes × 1790 slides)
[b] working day: based on 8-hour day
[c] Cytopathologist time calculation: 3.0 minutes per slide (3.0 minutes × 1790 slides)
[d] EBUS procedural time calculation: 15 minutes per biopsy site (15 minutes × 235 fewer sites).

Improved Laboratory Resource Utilization and Patient Care With the Use of Rapid On-Site Evaluation for Endobronchial Ultrasound Fine-Needle Aspiration Biopsy
Collins BT et al
Cancer (Cancer Cytopathol) 2013;121:544-51.

Matched case-control cohorts of TBNA with and without ROSE (340 each).

29.9% reduction in total slides

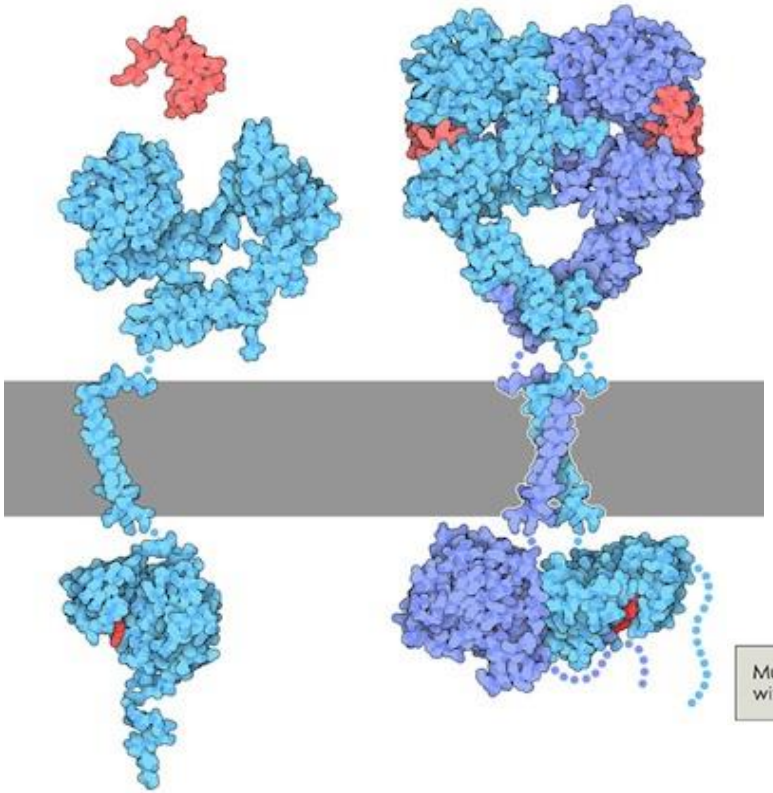
Savings in cytopathologist, BMS, procedure time

Process - mediastinum

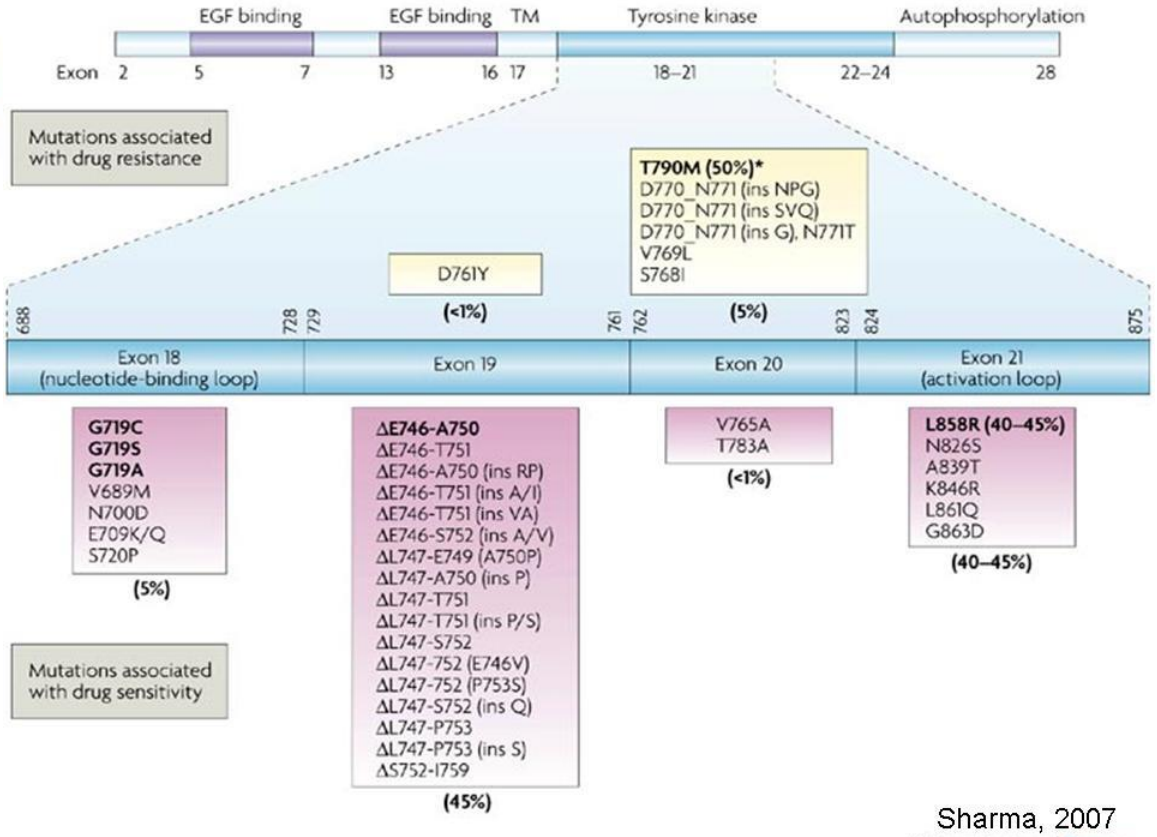
- Does ROSE help? - Yes
- Good evidence for reduction in sites with ROSE
- Limited evidence for reduction in passes/site
- Latter unsurprising due to
  - Time to stain and examine slides
  - Need for extra passes for ancillary studies
- In finance-driven health economies, may be savings

Mediastinum

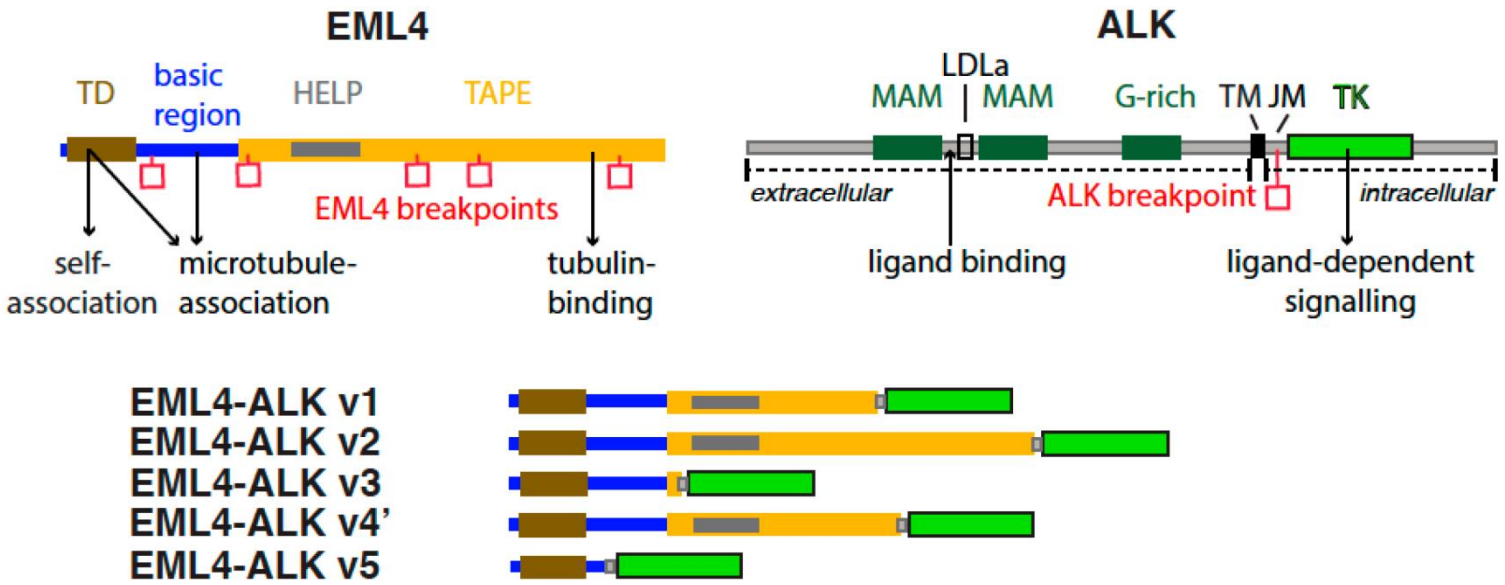
Ancillary tests



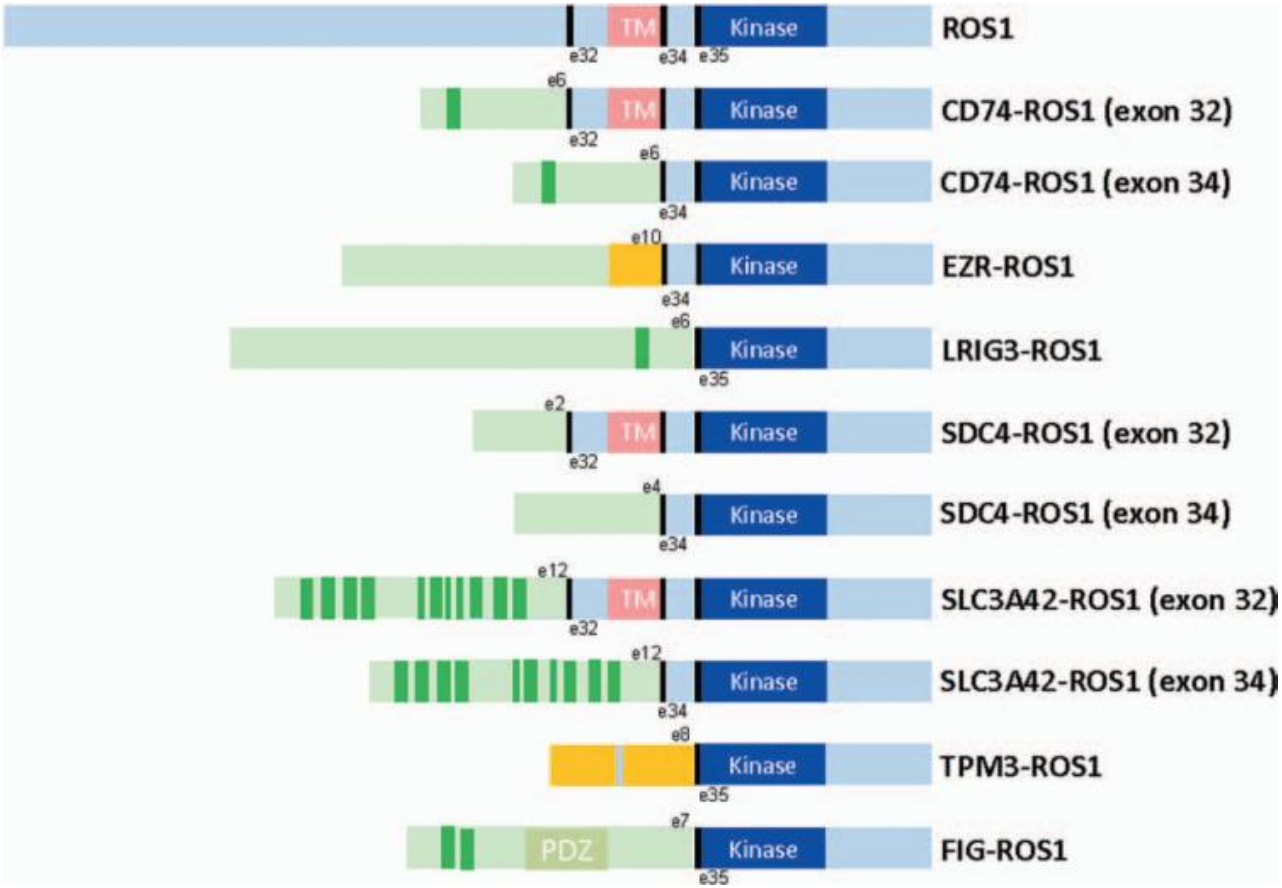
EGFR



Sharma, 2007
Nature Reviews | Cancer


EML4
TD
basic region
HELP
TAPE
EML4 breakpoints
self-association
microtubule-association
tubulin-binding
ALK
LDLa
MAM
MAM
G-rich
TM
JM
TK
extracellular
ALK breakpoint
intracellular
ligand binding
ligand-dependent signalling
EML4-ALK v1
EML4-ALK v2
EML4-ALK v3
EML4-ALK v4'
EML4-ALK v5

ALK


TM
Kinase
ROS1
e32
e34
e35
e6
CD74-ROS1 (exon 32)
CD74-ROS1 (exon 34)
e10
EZR-ROS1
LRIG3-ROS1
e2
SDC4-ROS1 (exon 32)
e4
SDC4-ROS1 (exon 34)
e12
SLC3A42-ROS1 (exon 32)
SLC3A42-ROS1 (exon 34)
e8
TPM3-ROS1
e7
PDZ
FIG-ROS1

ROS-1

PD-L1

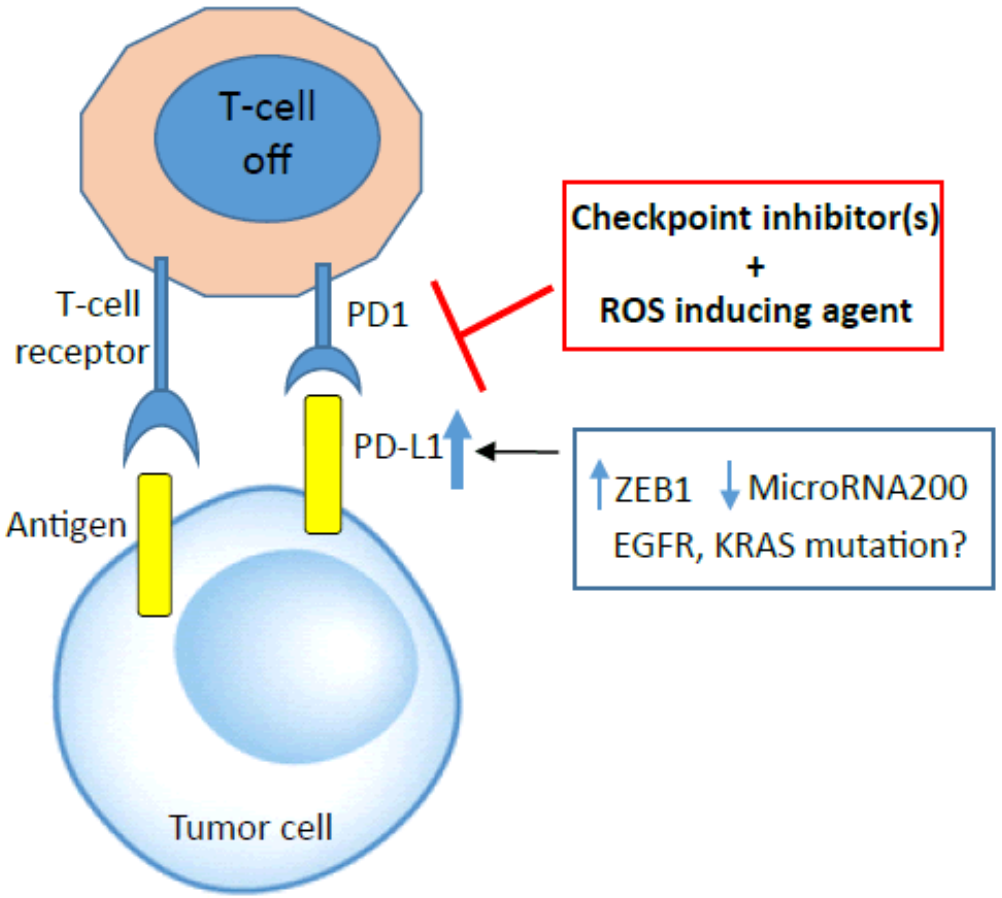


Figure 2: Cancers cells adapt and exploit immune system to evade immune surveillance by activating PD-L1/PD1 axis. ZEB1 and microRNA200 can regulate this axis. KRAS or EGFR mutation can also influence PD-L 1 expression. Blocking PD1 and PD-L1 interaction with checkpoint inhibitor(s) in combination with ROS inducing agent may lead to new approaches to overcome cisplatin resistant lung cancer.

Diagnostic molecular cytopathology

PRE–2004 PARADIGM			POST–2004 PARADIGM		
	CYTOLOGY	**HISTOLOGY**		**CYTOLOGY**	HISTOLOGY
ARCHITECTURAL FRAMEWORK	+	+++	ARCHITECTURAL FRAMEWORK	+	+++
CYTOLOGICAL DETAIL	+++	++/+++	CYTOLOGICAL DETAIL	+++	++/+++
QUALITY OF IHC	+/++	+++	QUALITY OF IHC	++/+++	+++
EASE OF SAMPLING	+++	+/++	EASE OF SAMPLING	+++	+/++
			MOLECULAR TESTING	+++	++/+++
CONCLUSION	In difficult / complex diagnosis, a preliminary cytology diagnosis should be followed by an histological confirmation		CONCLUSION	The molecular result becomes "pathognomonic" for diagnostics, or "final" for therapeutics – the cytology opinion does not need confirmation	

More Than a Decade of Molecular Diagnostic Cytopathology Leading Diagnostic and Therapeutic Decision-Making
Manuel Salto-Tellez, LMS/MD, FRCPath, FRCPI
Arch Pathol Lab Med—Vol 142, April 2018

Updates from 2016 Molecular Cytopathology meeting, Naples

Diagnostic molecular cytopathology

"Cytopathology is an integral part of the whole molecular revolution and, in some areas, such as molecular diagnostics of thyroid neoplasias or the therapeutic pathology of lung cancer, it is a leading application"

"Formalin-fixed, paraffin-embedded–based molecular testing, following adequate validation, can be applied to most cytopathology samples. Despite early attempts to deny that, it is now part of many national and international guidelines, including those in which cytopathology samples are a large fraction and those in which they may be an exception."

More Than a Decade of Molecular Diagnostic Cytopathology Leading Diagnostic and Therapeutic Decision-Making
Manuel Salto-Tellez, LMS/MD, FRCPath, FRCPI
Arch Pathol Lab Med—Vol 142, April 2018

Updates from 2016 Molecular Cytopathology meeting, Naples

ROSE – DNA quality from cell blocks

West Herts cases sent for NGS – January 2015 – March 2016

		DNA conc'n (ng/µl)		DIN	DIN allocation (cases)	
		Mean	Range	Mean	DIN<3	DIN>3
Cyto	EBUS/EUS (n=22; 21 for DIN)	8.73	0.82 - 40.4	4.29	5	16
	FNA (n=8)	4.23	0.76 – 19.8	1.46	6	2
	Pleural (n=5)	9.32	0.48 – 10.5	3.86	2	3
	Washings (n=2)	1.87	1.47 – 2.26	1.80	2	0
	Overall (n=37)	7.47	0.76 – 40.4	3.41	15	21
Histo	Core biopsy (n=14; 13 for DIN)	5.61	0.51 - 11.9	4.40	4	9
	Mucosal biopsy (n=8)	5.49	1.04 – 10.1	6.10	2	6
	Overall (n=22)	5.57	0.51 – 11.9	4.46	6	15

Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors

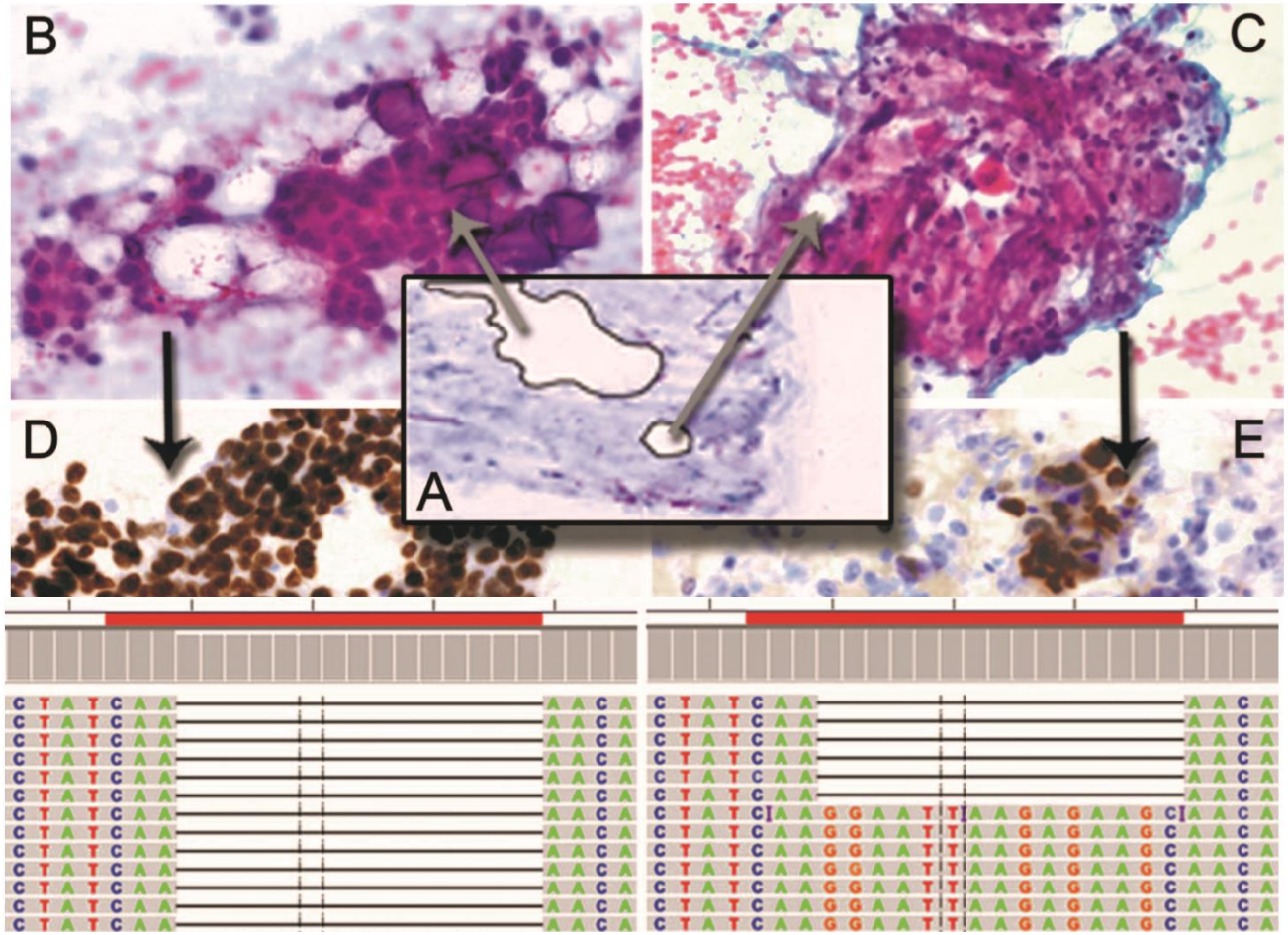
Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology

1. Any Cytology Sample With Adequate Cellularity and Preservation May Be Tested.—The original recommendation preferred cell blocks over smears. A recent systematic review[28] identified by the literature search has indicated that numerous studies have been published showing excellent performance of smear preparations, such that this preference is no longer appropriate. It is incumbent upon any laboratory that tests cytopathology specimens to perform appropriate validation studies of these as separate sample types, distinct from tissue and blood samples.

Arch Pathol Lab Med—Vol 142, March 2018

Cytology Smears in the Era of Molecular Biomarkers in Non–Small Cell Lung Cancer

Doing More With Less



Lozano et al, Arch Pathol Lab Med—Vol 142, March 2018

Does ROSE help with acquisition of tissue for molecular tests?

Guideline for the Acquisition and Preparation of Conventional and Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration Specimens for the Diagnosis and Molecular Testing of Patients with Known or Suspected Lung Cancer

Erik H.F.M. van der Heijden[a] Roberto F. Casal[b] Rocco Trisolini[c] Daniel P. Steinfort[d]
Bin Hwangbo[e] Takahiro Nakajima[f] Birgit Guldhammer-Skov[g] Giulio Rossi[h]
Maurizio Ferretti[i] Felix F.J. Herth[j] Rex Yung[k] Mark Krasnik[l]
on behalf of the World Association for Bronchology and Interventional Pulmonology
Task Force on Specimen Guidelines

Does ROSE influence tissue sampling for molecular analysis?

ROSE is very useful for the confirmation of the presence of tumor cells within the samples. Even though no prospective comparative trials have been published on the possible influence of ROSE on the diagnostic yield of TBNA or EBUS-TBNA for molecular testing, we suggest that ROSE be used when molecular testing is looked for until high-quality trials are available. Currently, an RCT aimed at evaluating the role of ROSE in EBUS-TBNA samples for molecular testing is ongoing (ClinicalTrials.gov identifier: NCT01799382).

Respiration 2014;88:500–517

TABLE 3] Results of the Study End Points

End Point	Overall Population (N = 197)			Patients With Nonsquamous NSCLC (n = 126)		
	ROSE (98)	EBUS (99)	*P* Value	ROSE (65)	EBUS (61)	*P* Value
Complete genotyping[a]	...	...	...	90.8	80.3	.094
Sensitivity[b]	97.5	95.1	.682	96.9	95.1	.673
Adequacy[b]	94.3	97.1	.357	94.9	97.7	.425

Data are presented as %. See Table 1 legend for expansion of abbreviations.
[a]Primary end point.
[b]Secondary end point.

Randomized Trial of Endobronchial UltrasoundGuided Transbronchial Needle Aspiration With and Without Rapid On-site Evaluation for Lung Cancer Genotyping

Trisolini et al

CHEST 2015; 148(6):1430-1437

197 patients randomised to EBUS TBNA with and without ROSE

Trend towards greater success in genotyping with ROSE but not statistically significant

Molecular testing on endobronchial ultrasound (EBUS) fine needle aspirates (FNA): Impact of triage

Simon Sung MD[1] | John P. Crapanzano MD[1] | David DiBardino MD[2] | David Swinarski PhD[3] | William A. Bulman MD[2] | Anjali Saqi MD, MBA[1]

Diagnostic Cytopathology. 2018;46:122–130.

TABLE 1 Key differences between Group A and B

	Group A	Group B
Triage *at start* of procedure	+	+/−
>1 cytology personnel	+	+/−
Slides prepared by clinical (non-cytology) staff	−	+/−

Retrospective analysis of 100 cases of lung adenocarcinoma in which EBUS with ROSE was utilised. Cases allocated to group A or B according to number and timing of cytology personnel

TABLE 1 Key differences between Group A and B

	Group A	Group B
Triage *at start* of procedure	+	+/−
>1 cytology personnel	+	+/−
Slides prepared by clinical (non-cytology) staff	−	+/−

There was a difference in availability of sufficient tissue for MT on cell blocks between Group A and Group B. One case from Group A (n = 1/22; 4.5%) and 20 from Group B (n = 20/78; 25.6%) had insufficient malignant cells in cell block(s) for MT. Because the smallest expected cell count in the resulting contingency table is smaller than 5, the classic Pearson-Fisher χ^2d test is not recommended for these data. Instead, following the recommendations of Campbell, the "$N-1$" $\chi^2 d$ test was used and showed that the difference between the rate of failure for MT in Group A and the rate of failure for MT in Group B is statistically significant with P values = 0.033.[21]

Retrospective analysis of 100 cases of lung adenocarcinoma in which EBUS with ROSE was utilised. Cases allocated to group A or B according to number and timing of cytology personnel

Does ROSE help with acquisition of tissue for molecular tests?

Yes, probably

Can you diagnose lymphoma at EBUS?

Endobronchial Ultrasound and Lymphoproliferative Disorders: A Retrospective Study

Seher Iqbal, MD, Zachary S. DePew, MD, Paul J. Kurtin, MD, Anne-Marie G. Sykes, MD, Geoffrey B. Johnson, MD, Eric S. Edell, MD, Thomas M. Habermann, MD, Fabien Maldonado, MD

The Annals of Thoracic Surgery
Volume 94, Issue 6, Pages 1830-1834 (December 2012)

- Mayo Clinic: 2006-2011
- Retrospective study cross-referencing lymphoma + EBUS databases
- 65 patients
- Sensitivity 29%
  - 21G needle
  - No ROSE – min 3 passes, unless 2 passes produced visible core
  - No flow cytometry

Can you diagnose lymphoma at EBUS?

Diagnosis and Subtyping of De Novo and Relapsed Mediastinal Lymphomas by Endobronchial Ultrasound Needle Aspiration

Mufaddal T. Moonim, Ronan Breen, Paul A. Fields, and George Santis

Am J Respir Crit Care Med Vol 188, Iss. 10, pp 1216–1223, Nov 15, 2013

- 100 cases of suspected lymphoma in 5 years
  - ROSE service
  - Flow cytometry + cytogenetics etc available
- Correct diagnosis of
  - 48/51 de novo lymphoma (88%)
  - 15/15 relapsed lymphoma (100%)
  - 32/34 non-lymphoma (96%)
- Sensitivity/specificity = 89%/97%
- Sensitivity of sub-typing
  - HGL – 90%
  - LGL – 100%
  - HD – 79%
- EBUS result enough for clinical mgt in 84/100 (84%)

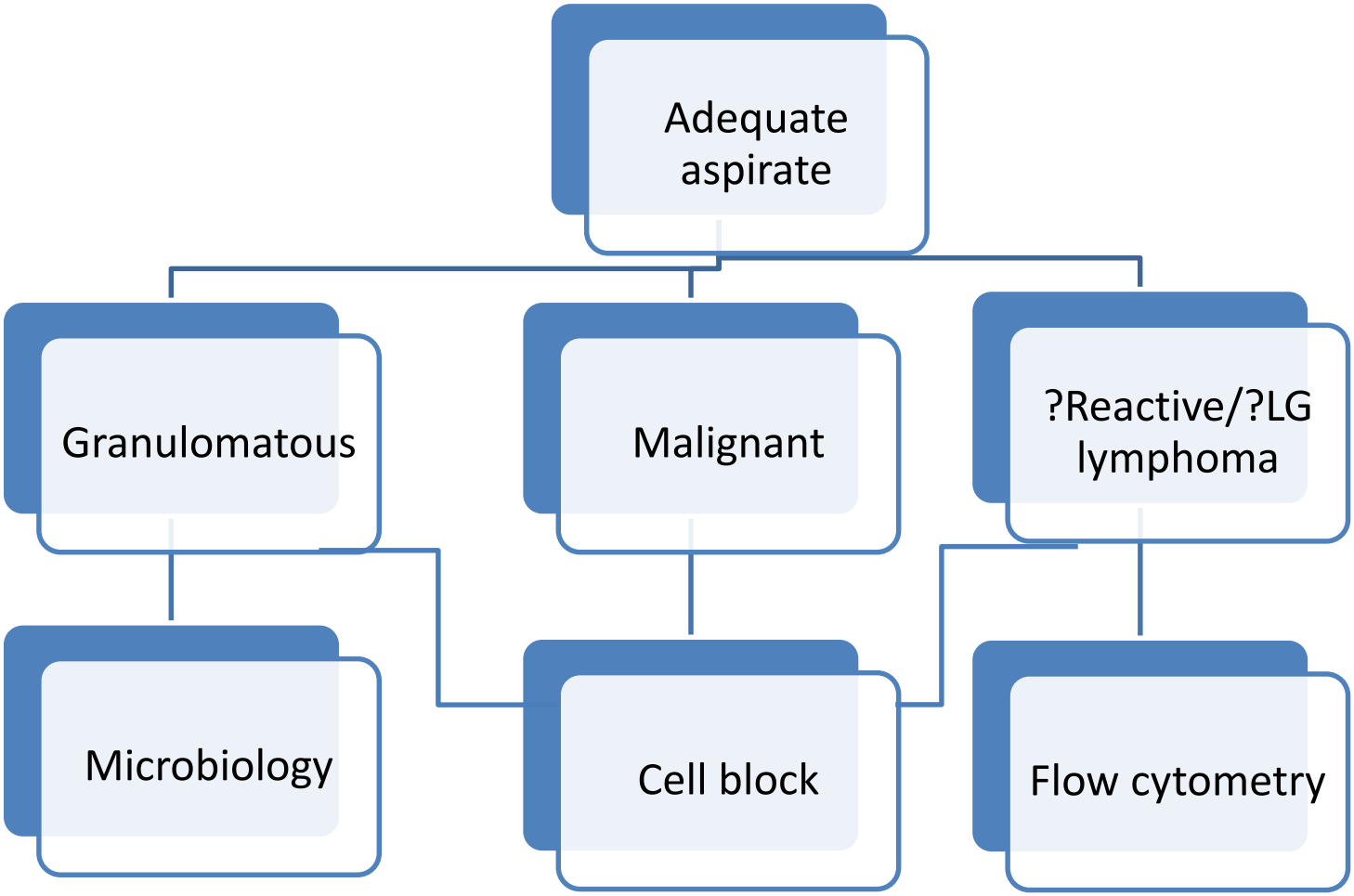
Can you diagnose lymphoma at EBUS?

Yes, but there needs to be

- Good (ie abundant) cell block material
- Appropriate material for flow cytometry, if necessary
- A good relationship with the Haematopathology service, wherever that is
  - Specialist Integrated Haematological Malignancy Diagnostic Service
- And the sensitivity for HD and HGNHL may be a challenge

West Hertfordshire Hospitals NHS
NHS Trust

ROSE – specimen management



ROSE in the mediastinum – summary

- Advantages
  - Instant (actually 2-3 minute) feedback for endoscopist
    - Adequacy and provisional diagnosis
  - Specimen management and triage
    - Solid tumour/high grade lymphoma – cell block
    - ?Reactive node/?low grade lymphoma – flow cytometry
    - Granulomas – microbiology
  - Reduction of sites/patient (?passes/site)
- Disadvantages/reasons not more utilised
  - BMS and/or consultant time and resource
  - May be out of comfort zone for either
  - Potential specimen compromise – endoscopist's fear of slides+++++/insufficient material for molecular

Pancreas

Pancreatic EUS – key differences

- Generally only one target
  - Though possible to sample lymph nodes as well
- Clear division into
  - Solid and cystic lesions
  - Different sample handling and implications
- For the majority of solid pancreatic lesions (ie pancreatic ductal carcinoma), diagnosis is morphological

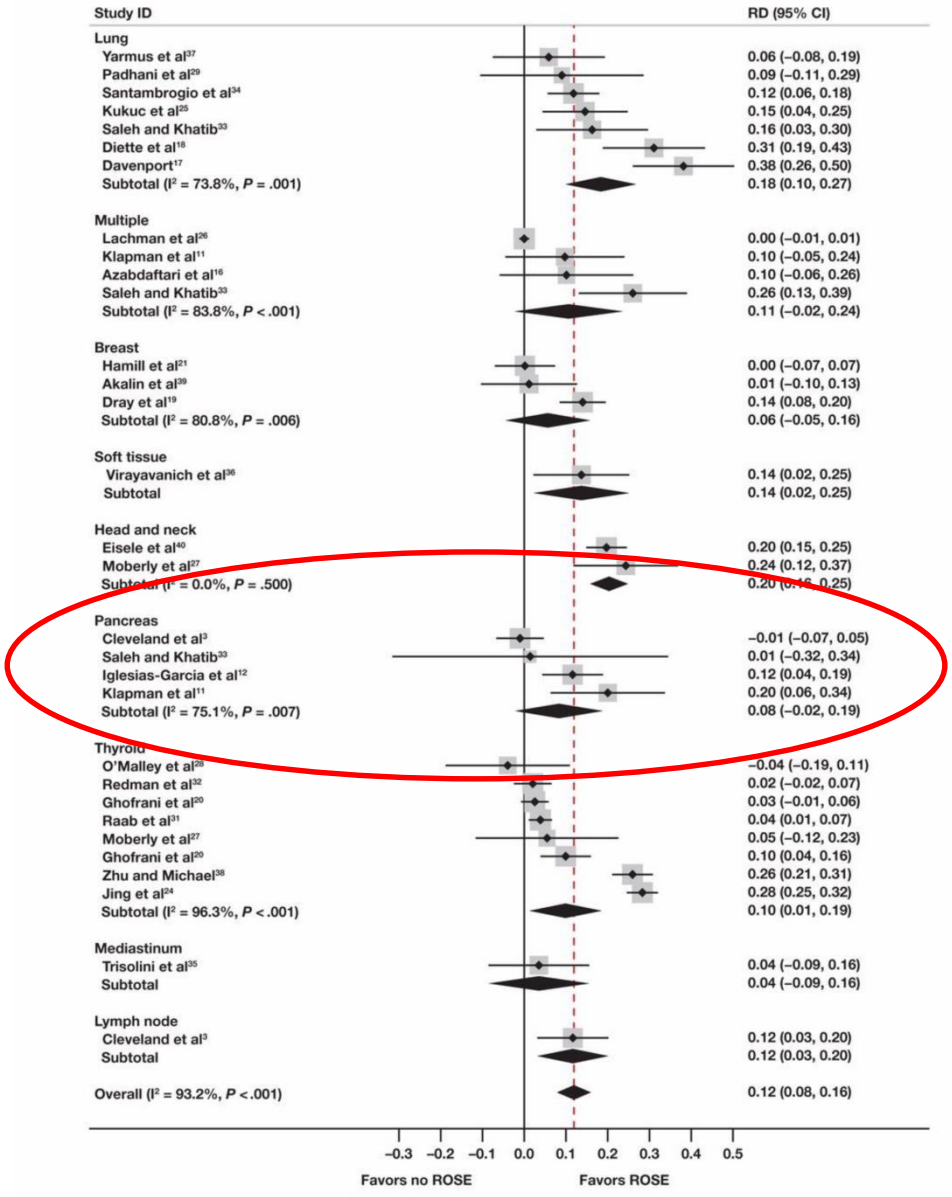
Pancreatic EUS – key challenges

- GI epithelial contamination is a major issue
- Benign inflammatory lesions are a problem (IgG4, chronic pancreatitis)
- Specimens may be paucicellular

Pancreatic EUS

- So, is there any point doing ROSE, if
  - You can't lower the number of targeted sites, and
  - Ancillary tests are less used?

- Well, there's always adequacy, diagnostic yield, process etc.



The Influence of Rapid Onsite Evaluation on the Adequacy Rate of Fine-Needle Aspiration Cytology. A Systematic Review and Meta-Analysis.

Schmidt RL et al

Am J Clin Pathol. 2015;139(3):300-308.
doi:10.1309/AJCPEGZMJKC42VUP

Meta-analysis of 25, 2-cohort, studies with and without ROSE, a total of 12,407 cases

Forest plot shows change in adequacy rate when ROSE used. Analysis is not adjusted for initial adequacy.

Equation Factor	Coefficient		ROSE Impact	95% CI		t^b	P
	Symbol	Value		Lower	Upper		
Non-ROSE adequacy rate, X_j	β	–0.67		–0.82	–0.51	–8.91	<.001
Tissue effects							
Breast	α_1	0.00	Low	Reference			
Pancreas	α_2	0.14	High	0.05	0.23	3.26	.004
Lung	α_3	0.14	High	0.07	0.22	3.78	.001
Lymph node	α_4	0.15	High	0.02	0.28	2.42	.02
Thyroid	α_5	0.14	High	0.07	0.21	4.07	<.001
Multiple	α_6	0.12	High	0.03	0.20	2.81	.01
Mediastinum	α_7	0.01	Low	–0.15	0.16	0.12	.90
Soft tissue	α_8	0.03	Low	–0.12	0.18	0.44	.66
Head and neck	α_9	0.15	High	0.05	0.24	3.28	.004
Constant	κ	0.53		0.40	0.65	9.00	<.001

The Influence of Rapid Onsite Evaluation on the Adequacy Rate of Fine-Needle Aspiration Cytology. A Systematic Review and Meta-Analysis.

Schmidt RL et al

Am J Clin Pathol. 2015;139(3):300-308.
doi:10.1309/AJCPEGZMJKC42VUP

Meta-analysis of 25, 2-cohort, studies with and without ROSE, a total of 12,407 cases

Analysis adjusted for initial adequacy.

The presence of a cytopathologist increases the diagnostic accuracy of endoscopic ultrasound-guided fine needle aspiration cytology for pancreatic adenocarcinoma: a meta-analysis

S. Hébert-Magee*, S. Bae†, S. Varadarajulu‡, J. Ramesh‡, A. R. Frost*, M. A. Eloubeidi‡ and I. A. Eltoum*

*Division of Anatomic Pathology, Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, USA, †Division of Preventive Medicine, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA, ‡Division of Gastroenterology and Hepatology, Department of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

Cytopathology 2013, **24**, 159–171

Table 5. Predefined subgroup analysis with multivariate meta-regression showing only cytopathology is statistically significant

Subgroup	RDOR 95% (CI)	*P*-value
Number of patients	1.00 (1.00–1.01)	0.1329
On-site cytopathology	5.95 (2.15–16.45)	0.0012
Reference standard	4.91 (0.62–38.92)	0.1264

Meta-analysis of 34 studies (3644 patients) some with, some without ROSE. No effect on adequacy, but diagnostic accuracy improves with ROSE.

Rapid On-Site Evaluation Does Not Improve Endoscopic Ultrasound-Guided Fine Needle Aspiration Adequacy in Pancreatic Masses: A Meta-Analysis and Systematic Review

Fanyang Kong[1☯], Jianwei Zhu[1☯], Xiangyu Kong[1☯], Tao Sun[1], Xuan Deng[2], Yiqi Du[1‡*], Zhaoshen Li[1‡*]

1 Department of Gastroenterology, Changhai Hospital, Second Military Medical University, Shanghai, China, **2** Shanghai Medical College of Fudan University, Shanghai, China

	ROSE		Without ROSE			Risk Difference	
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Random, 95% CI	Year
Alsohaibani2009	14	22	14	22	7.2%	0.00 [-0.28, 0.28]	2009
Garcia2011	92	95	67	87	22.2%	0.20 [0.10, 0.29]	2011
Cermak2012	124	167	162	214	23.1%	-0.01 [-0.10, 0.07]	2012
Nayar2013	83	97	73	82	21.9%	-0.03 [-0.13, 0.06]	2013
Wani2015	114	121	108	120	25.5%	0.04 [-0.03, 0.11]	2015
Total (95% CI)		**502**		**525**	**100.0%**	**0.04 [-0.04, 0.13]**	
Total events	427		424				

Heterogeneity: Tau² = 0.01; Chi² = 14.65, df = 4 (P = 0.005); I² = 73%
Test for overall effect: Z = 0.99 (P = 0.32)

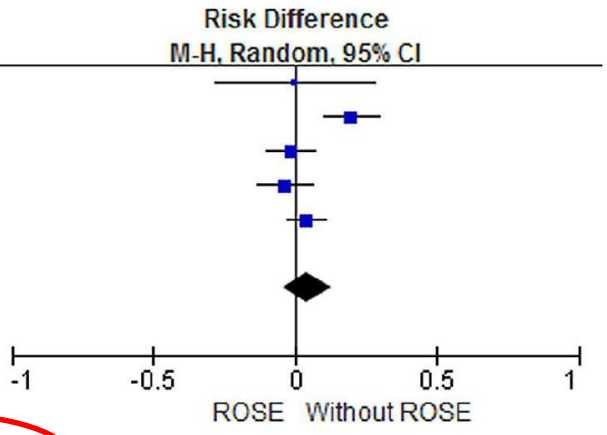


Fig 4. Forest plot displaying the Risk Difference and 95% CIs of each study for the diagnosis yield.

PLOS ONE | DOI:10.1371/journal.pone.0163056 September 22, 2016

Meta-analysis of 7 studies (1299 patients)

Rapid On-Site Evaluation for Endoscopic Ultrasound-Guided Fine-Needle Biopsy of the Pancreas Decreases the Incidence of Repeat Biopsy Procedures

Brian T. Collins, MD[1]; Faris M. Murad, MD[2]; Jeff F. Wang, MD[1]; and Cory T. Bernadt, MD, PhD[1]

TABLE 2. ROSE EUS FNA Biopsy in Repeat Procedures: Proportional Difference

Biopsy	Repeat Patients/ All Patients	Proportional Ratio
Non-ROSE service	22/377	0.0584
ROSE service	11/379	0.029
Difference	50% difference	−0.0293 (*P* value <.024)

Abbreviations: EUS FNA, endoscopic ultrasound-guided fine-needle aspiration; ROSE, rapid on-site evaluation.

TABLE 4. ROSE EUS FNA Biopsy in Repeat Procedures: Definitive Categorization After Second Biopsy

Biopsy	Definitive Diagnosis on Second Biopsy/All Patients	Proportional Ratio
Non-ROSE service	6/22 (27%)	0.273
ROSE service	7/11 (64%)	0.636
Difference	37% higher rate of positivity on ROSE second biopsy Twice as likely to have a definitive positive using ROSE than non-ROSE service	0.364 (*P* value <.044)

Case-controlled cohort study, 377 non-ROSE, 379 ROSE

(Cancer Cytopathol) 2013;121:518–24.

Does ROSE help with pancreatic EUS?

Maybe(with adequacy and diagnostic yield)

Pancreas – ancillary tests

- Immunocytochemistry
  - For the minority of solid lesions that are not pancreatic ductal carcinoma, immuno may be crucial – ie cell blocks needed
- Molecular - currently
  - No guidelined role for molecular testing in solid pancreatic lesions
  - However, there is a role for KRAS testing in cystic pancreatic lesions – distinguishes lesions of mucinous origin
- Other ancillary tests
  - CEA/amylase in cyst fluid in ddx of pseudocyst/mucinous cyst

Head and neck

Equation Factor	Coefficient Symbol	Coefficient Value	ROSE Impact	95% CI Lower	95% CI Upper	t^b	*P*
Non-ROSE adequacy rate, X_j	β	–0.67		–0.82	–0.51	–8.91	<.001
Tissue effects							
Breast	α_1	0.00	Low	Reference			
Pancreas	α_2	0.14	High	0.05	0.23	3.26	.004
Lung	α_3	0.14	High	0.07	0.22	3.78	.001
Lymph node	α_4	0.15	High	0.02	0.28	2.42	.02
Thyroid	α_5	0.14	High	0.07	0.21	4.07	<.001
Multiple	α_6	0.12	High	0.03	0.20	2.81	.01
Mediastinum	α_7	0.01	Low	–0.15	0.16	0.12	.90
Soft tissue	α_8	0.03	Low	–0.12	0.18	0.44	.66
Head and neck	α_9	0.15	High	0.05	0.24	3.28	.004
Constant	κ	0.53		0.40	0.65	9.00	<.001

The Influence of Rapid Onsite Evaluation on the Adequacy Rate of Fine-Needle Aspiration Cytology. A Systematic Review and Meta-Analysis.

Schmidt RL et al

Am J Clin Pathol. 2015;139(3):300-308.
doi:10.1309/AJCPEGZMJKC42VUP

Meta-analysis of 25, 2-cohort, studies with and without ROSE, a total of 12,407 cases

Analysis is adjusted for initial adequacy.

2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer

"The largest studies of preoperative molecular markers in patients with indeterminate FNA cytology have respectively evaluated a seven-gene panel of genetic mutations and rearrangements (*BRAF*, *RAS*, *RET/PTC*, *PAX8/PPARγ*), a gene expression classifier (167 GEC; mRNA expression of 167 genes), and galectin-3 immunohistochemistry (cell blocks)."

[A17] AUS/FLUS cytology
■ RECOMMENDATION 15

For nodules with AUS/FLUS cytology, after consideration of worrisome clinical and sonographic features, investigations such as repeat FNA or molecular testing may be used to supplement malignancy risk assessment in lieu of proceeding directly with a strategy of either surveillance or diagnostic surgery….

In summary, there is currently no single optimal molecular test that can definitively rule in or rule out malignancy in all cases of indeterminate cytology, and long-term outcome data proving clinical utility are needed.

[A19] Suspicious for malignancy cytology
■ RECOMMENDATION 17

(B) After consideration of clinical and sonographic features, mutational testing for *BRAF* or the seven-gene mutation marker panel (*BRAF*, *RAS*, *RET/PTC*, *PAX8/PPARγ*) may be considered in nodules with SUSP cytology if such data would be expected to alter surgical decision-making.

- Molecular testing in thyroid disease is not yet mandated but…
- It would be wise to make sure you have a robust mechanism ready for molecular testing of your thyroid specimens in the future…

Head and neck - summary

- Reasonable evidence that ROSE improves adequacy and diagnostic yield
- Ancillary tests similar to other sites – immuno for selected cases, flow and immuno for possible lymphoma, micro for possible infection
- Molecular testing in thyroid a fast-developing field

ROSE in West Herts – preparations

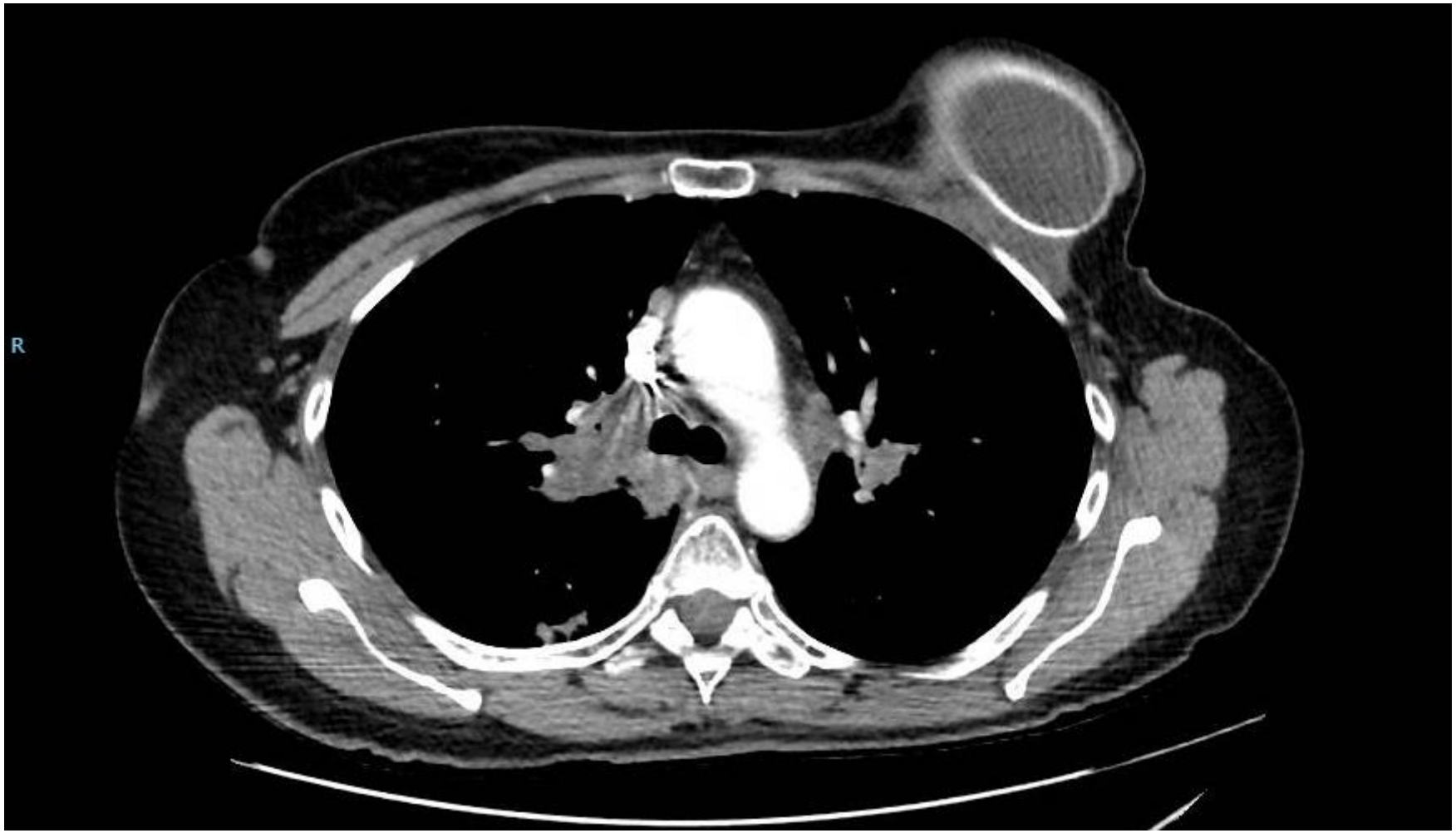
- 3 slides per pass
  - one air-dried - rapid-stained for ROSE
  - one fixed for later Pap stain
  - one “spreader” air-dried – later MGG
- Solid material into formalin for cell block
- “Bloody” material into saline for cell block later
- Micro – sterile saline
- Flow – saline flush then into EDTA tube

- If ROSE team cannot attend – all into ThinPrep (LBC) unless lymphoma/infection suspected

ROSE at West Herts

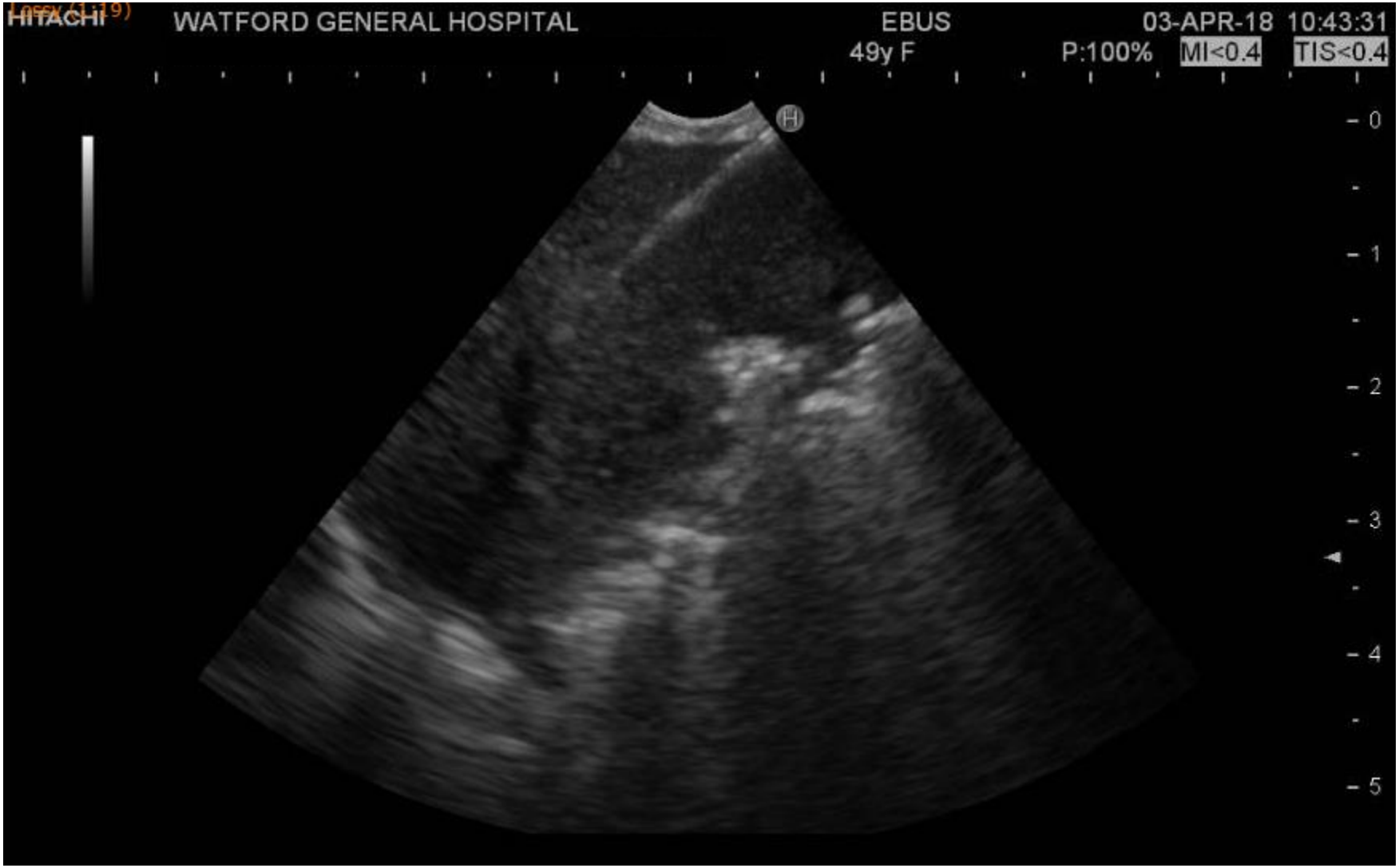
- 49 year old woman
- Aug 2016 – G3 IDC, ER 0, PR 3, HER2 3+
  - Rx primary chemo + Herceptin
- MRI – 3 x residual foci of carcinoma
- Mastectomy April 2017
- February 2018 – cough
- CT showed R hilar mass - EBUS

ROSE at West Herts



ROSE at West Herts

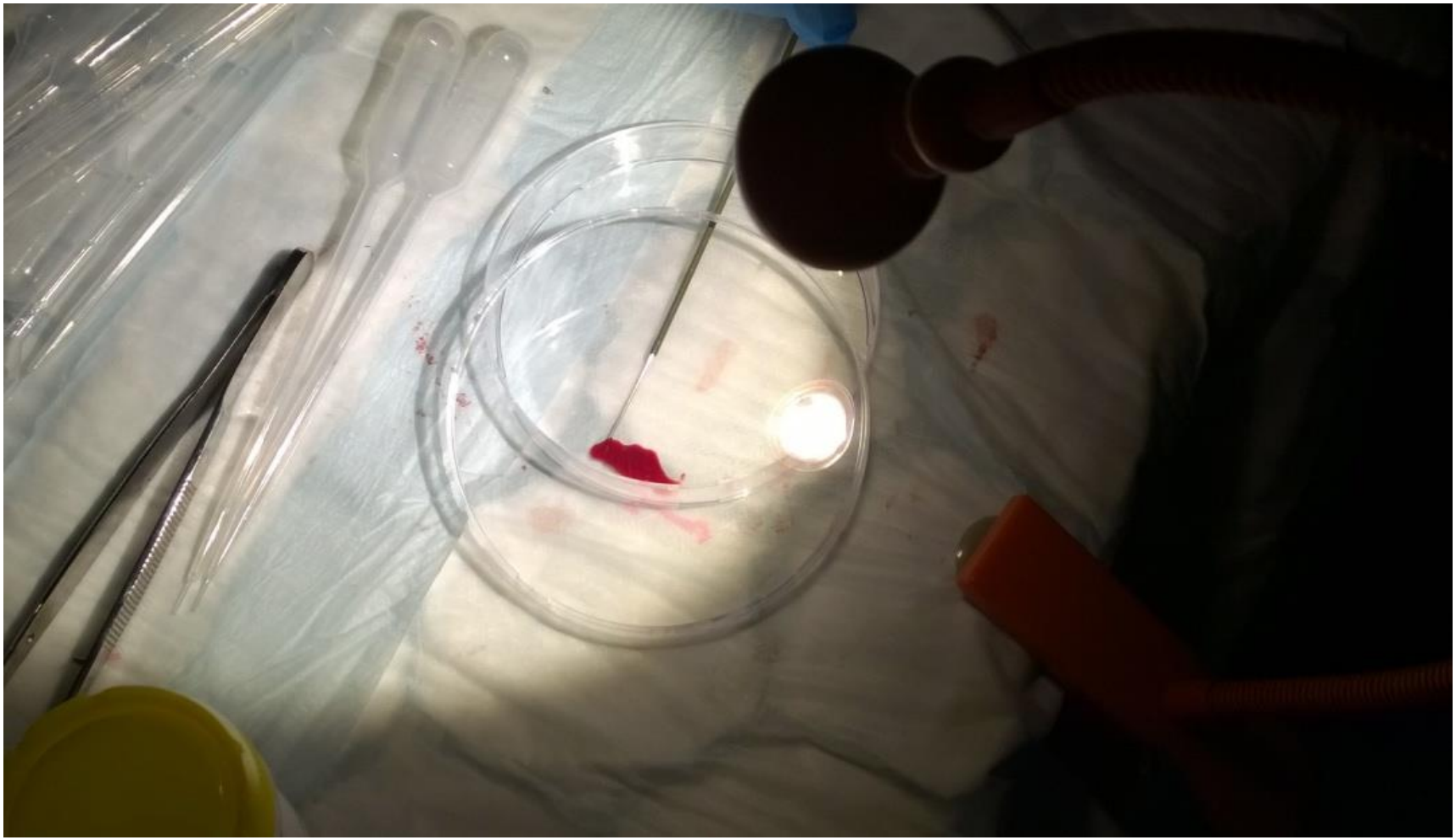
West Hertfordshire Hospitals NHS
NHS Trust



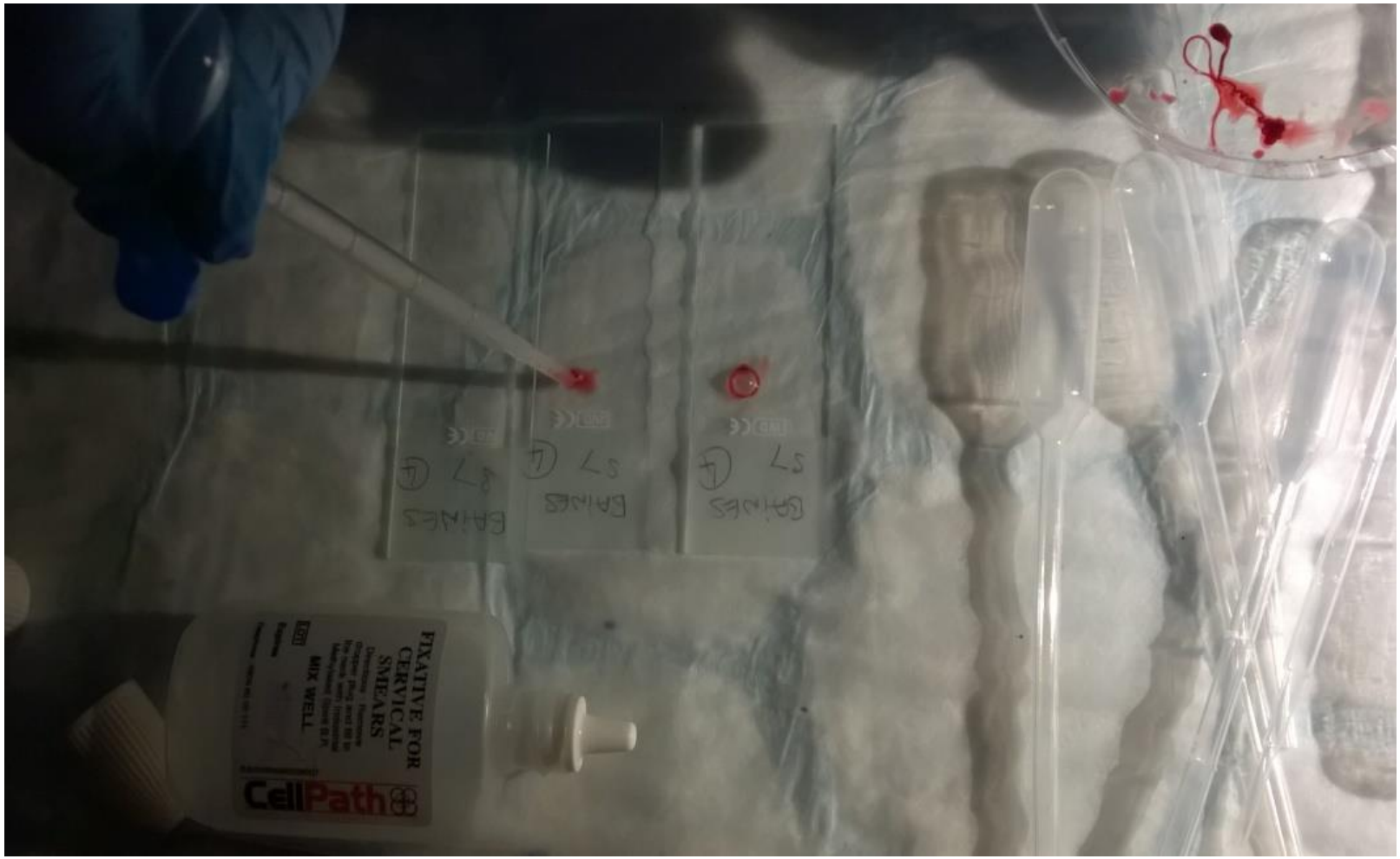
ROSE at West Herts

West Hertfordshire Hospitals NHS
NHS Trust

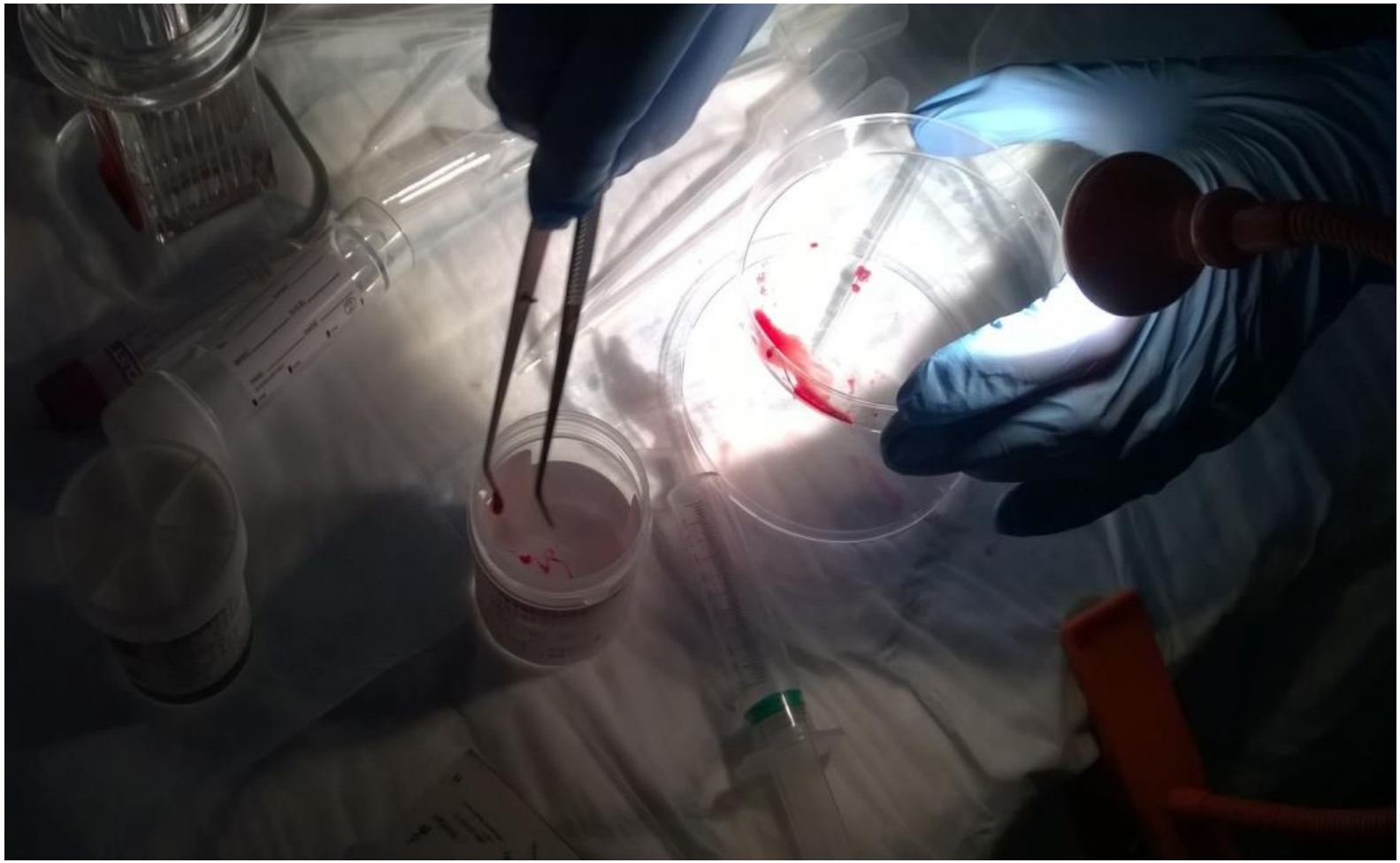
ROSE at West Herts



ROSE at West Herts

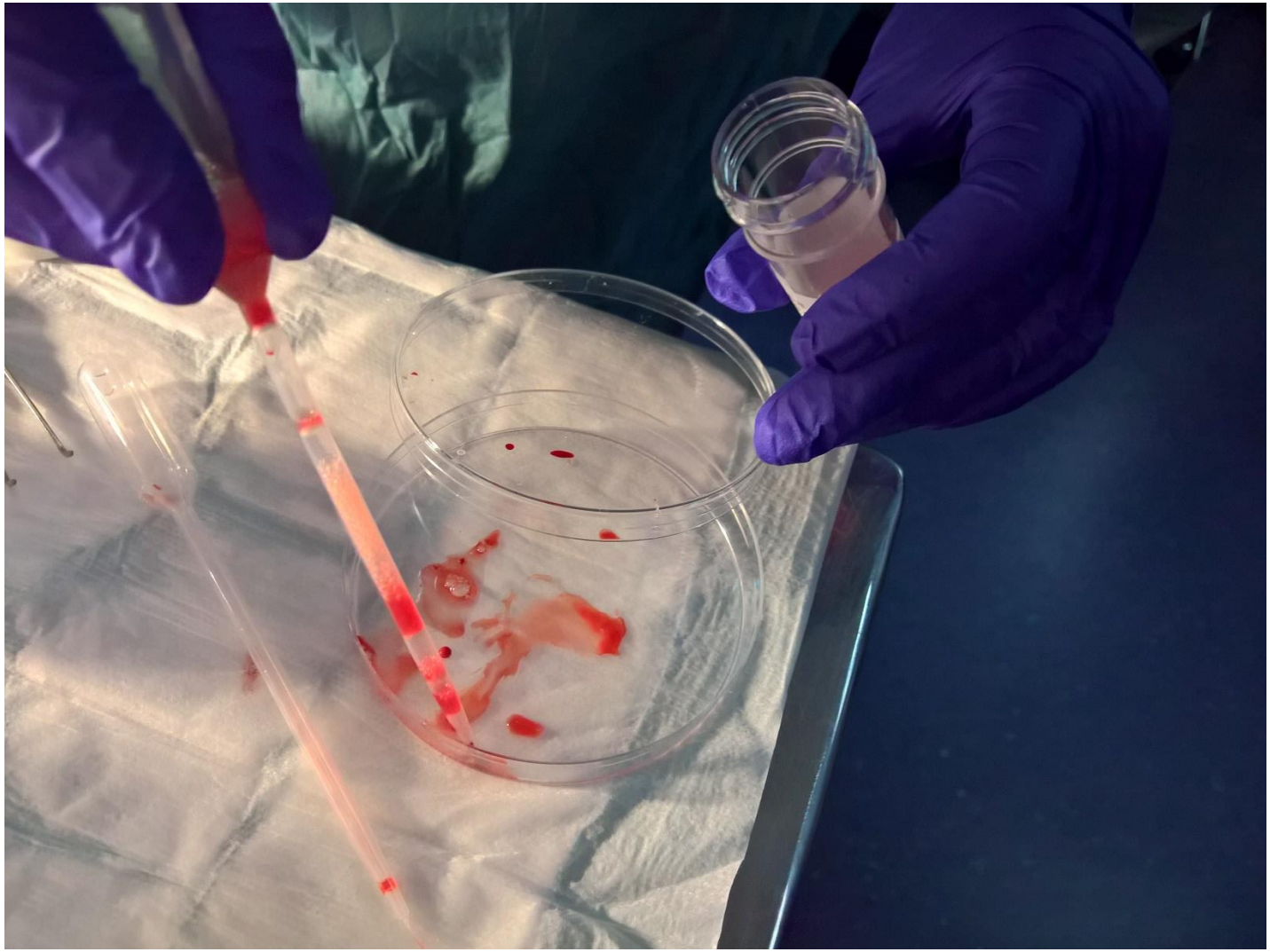


ROSE at West Herts



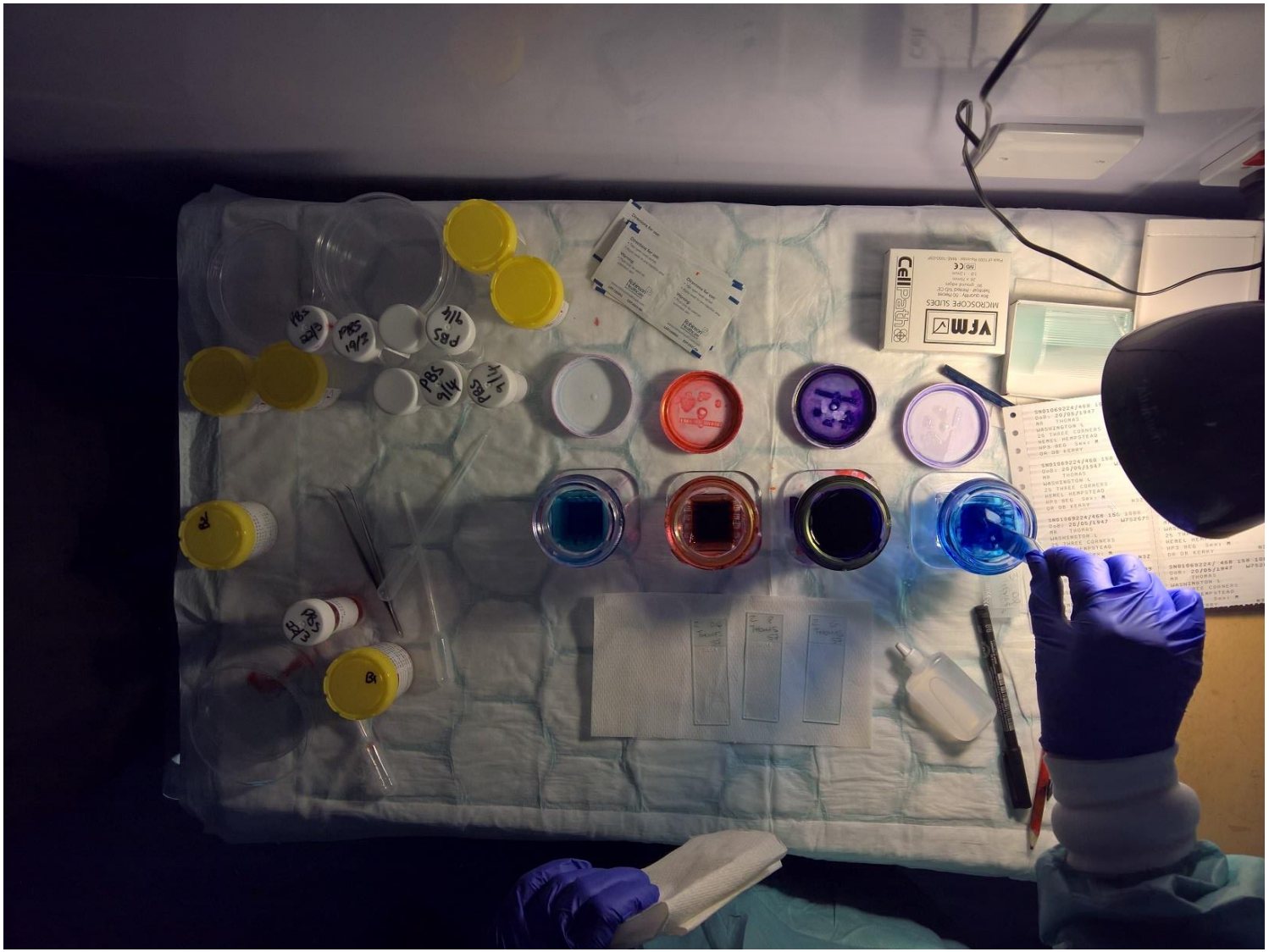
ROSE at West Herts

West Hertfordshire Hospitals NHS
NHS Trust



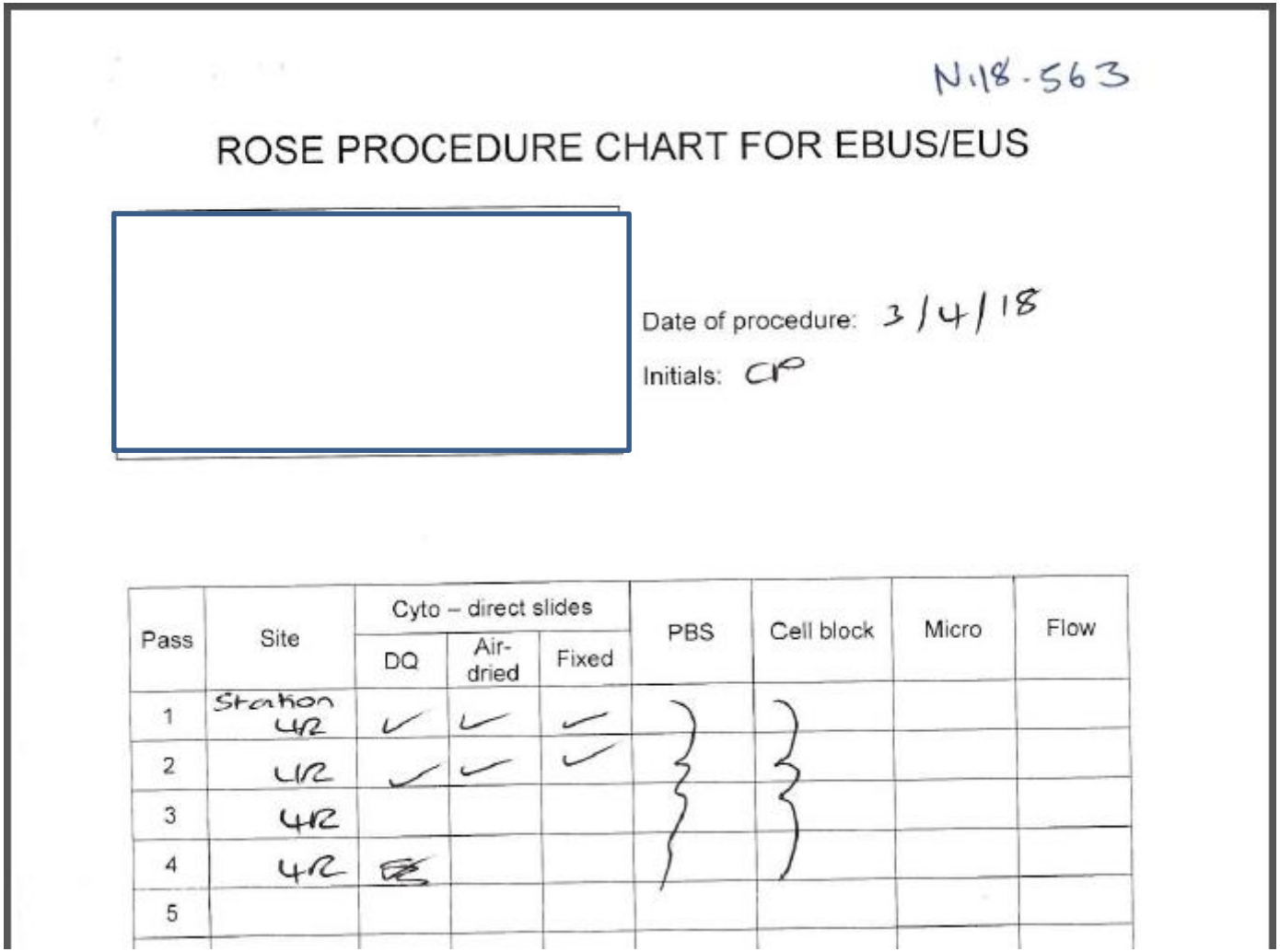
ROSE at West Herts

West Hertfordshire Hospitals NHS
NHS Trust



ROSE at West Herts

ROSE at West Herts



N:18-563

ROSE PROCEDURE CHART FOR EBUS/EUS

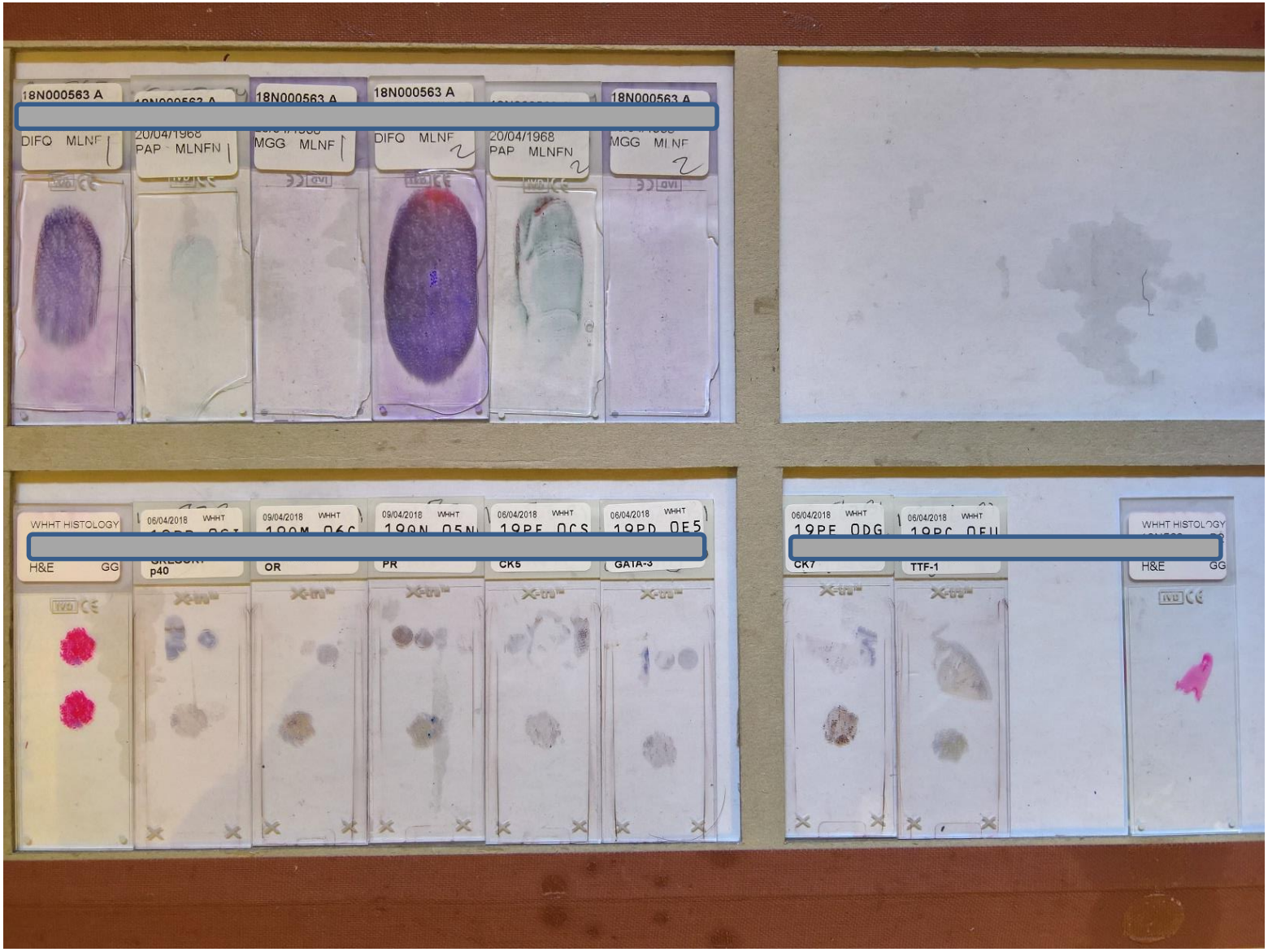
Date of procedure: 3/4/18

Initials: CP

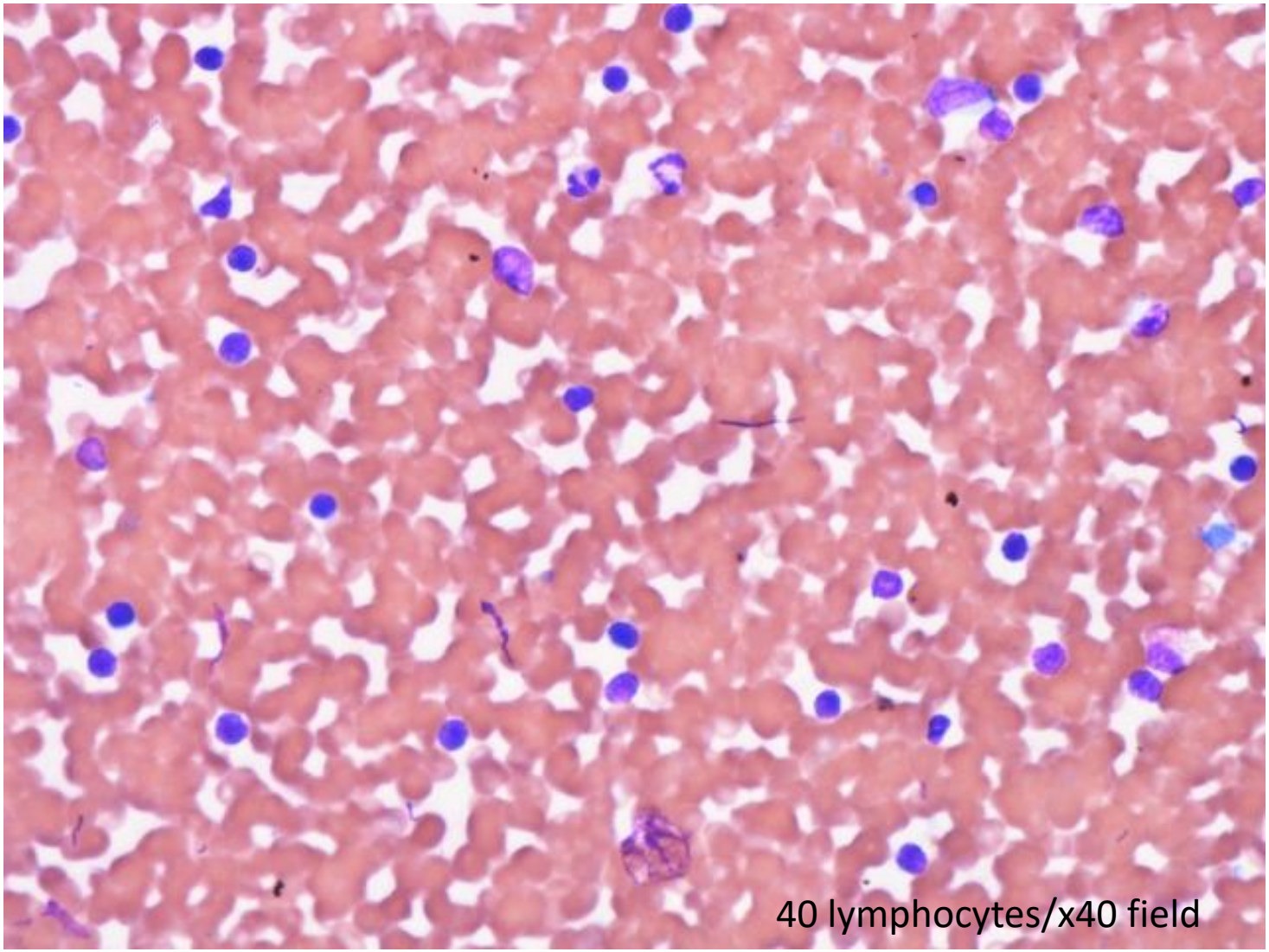
Pass	Site	Cyto – direct slides			PBS	Cell block	Micro	Flow
		DQ	Air-dried	Fixed				
1	Station 4R	✓	✓	✓				
2	4R	✓	✓	✓				
3	4R							
4	4R	✓						
5								

ROSE at West Herts

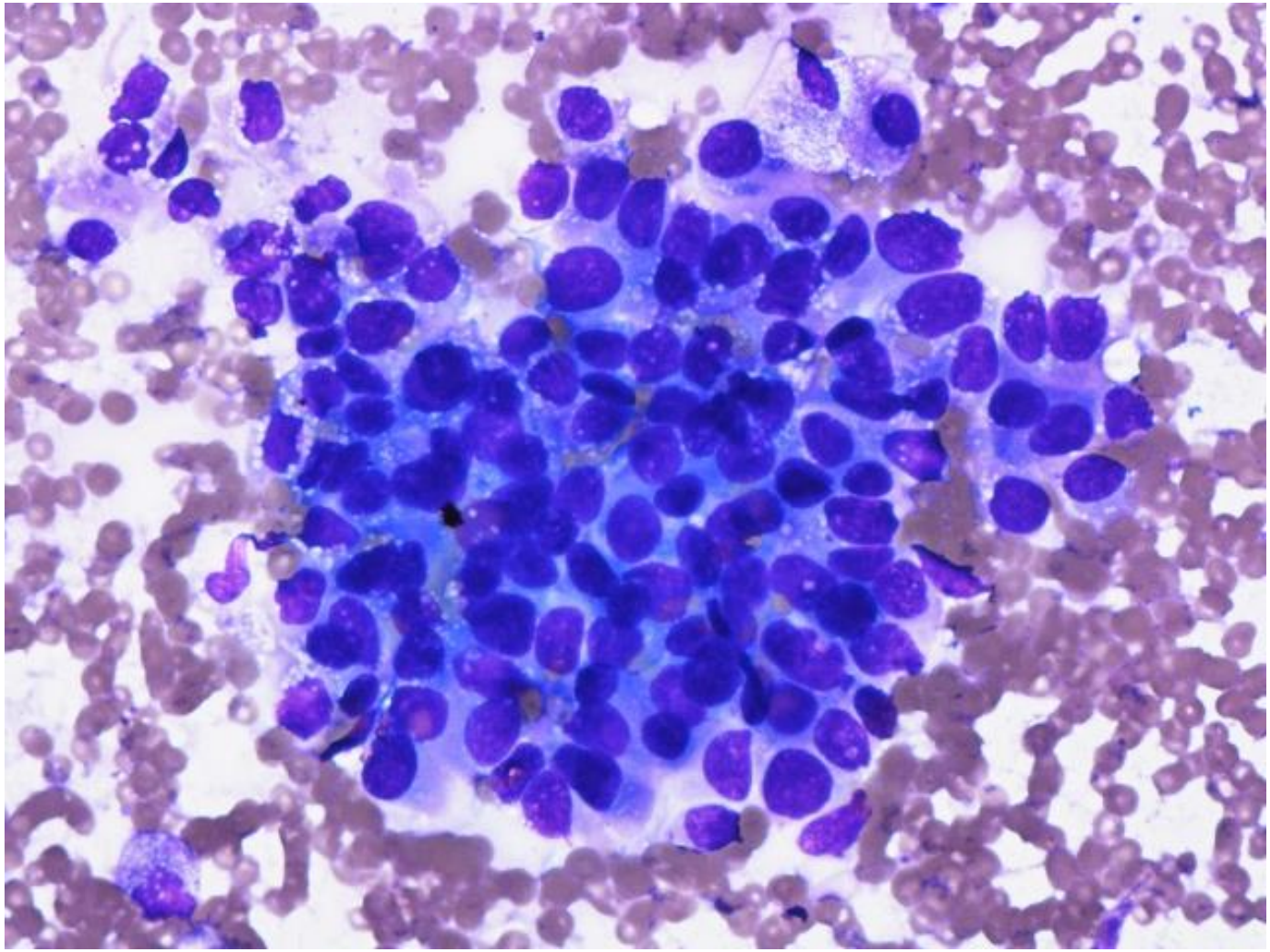
ROSE at West Herts



ROSE at West Herts

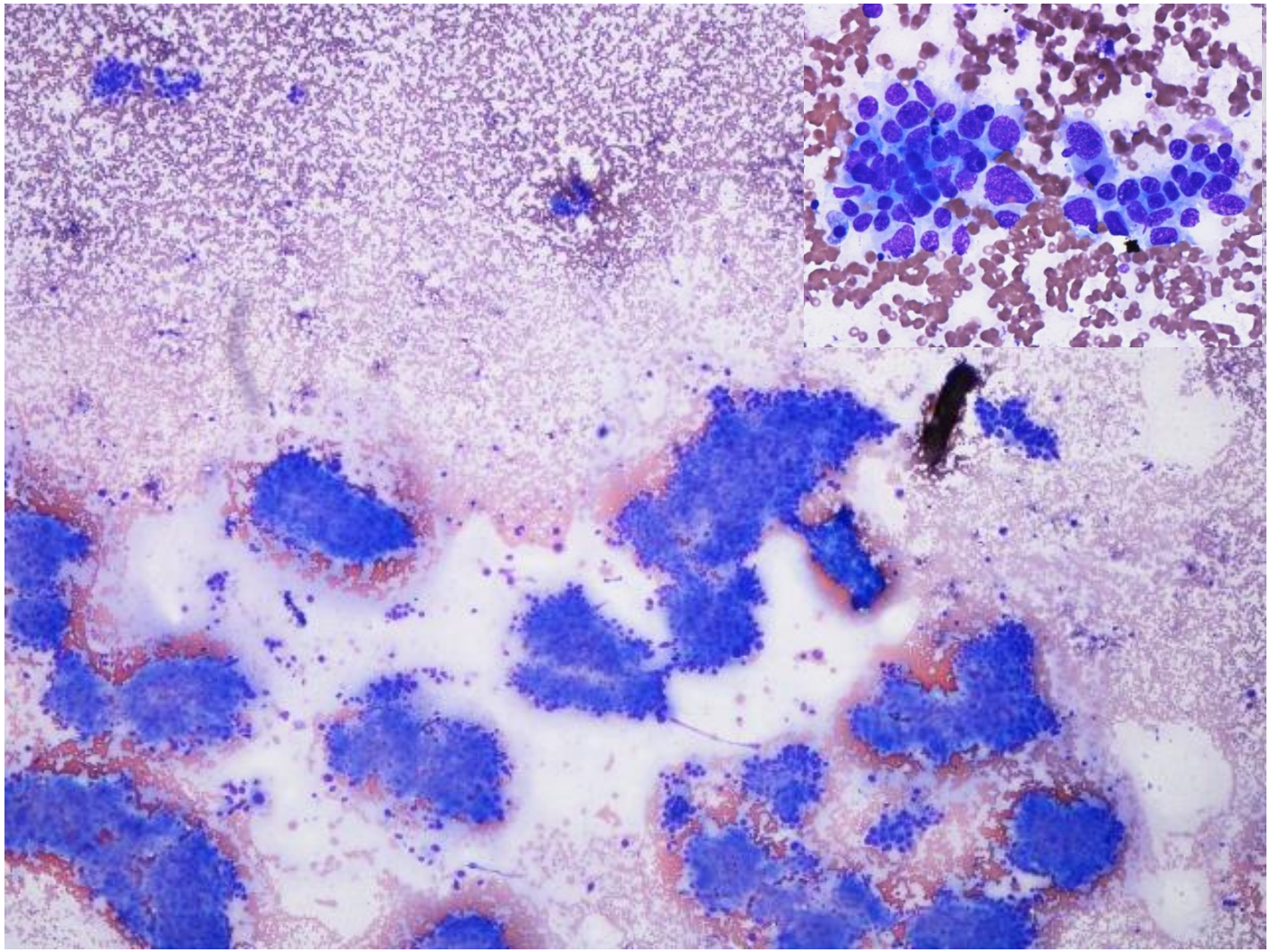


ROSE at West Herts



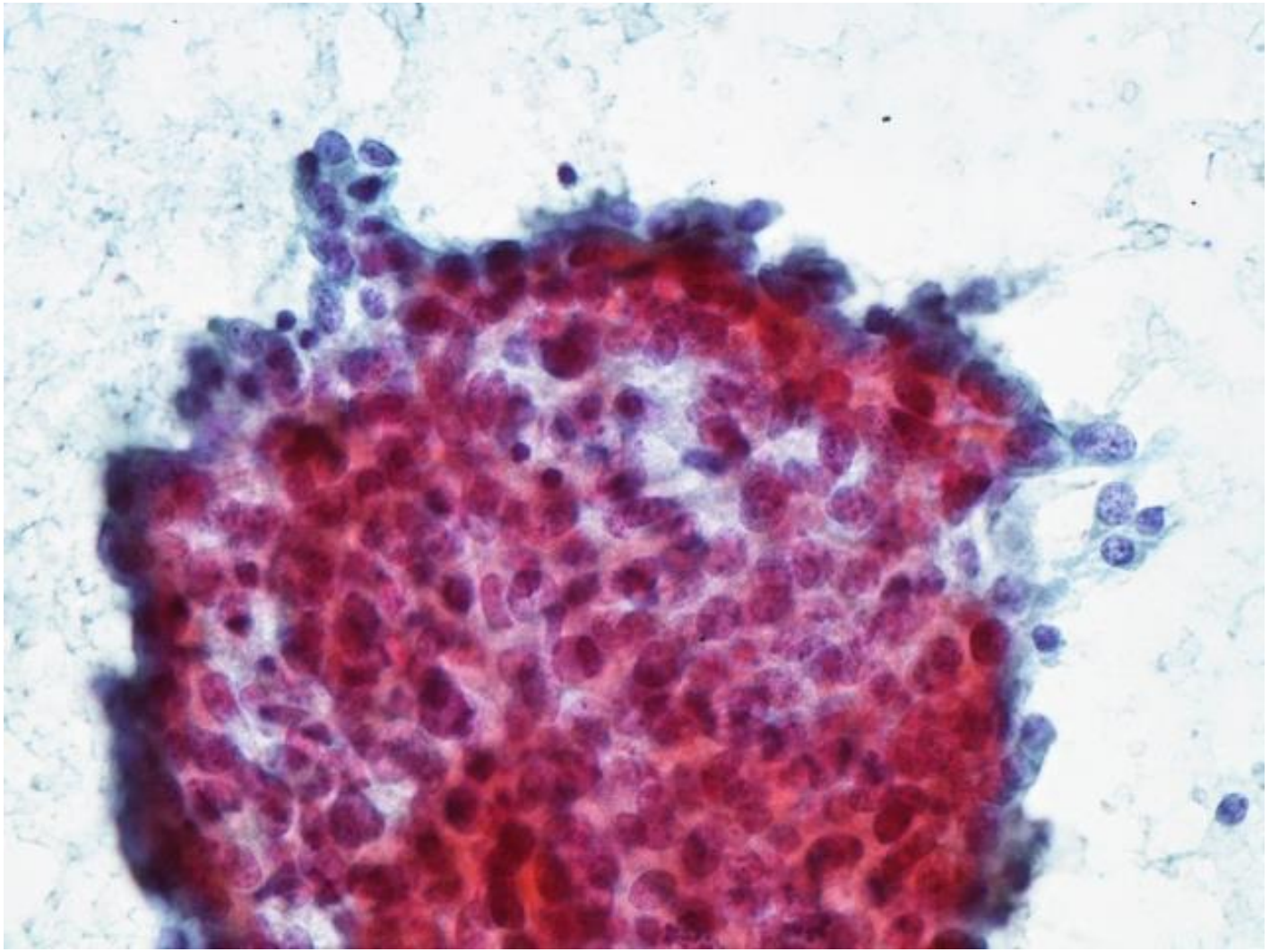
Pass 1

ROSE at West Herts



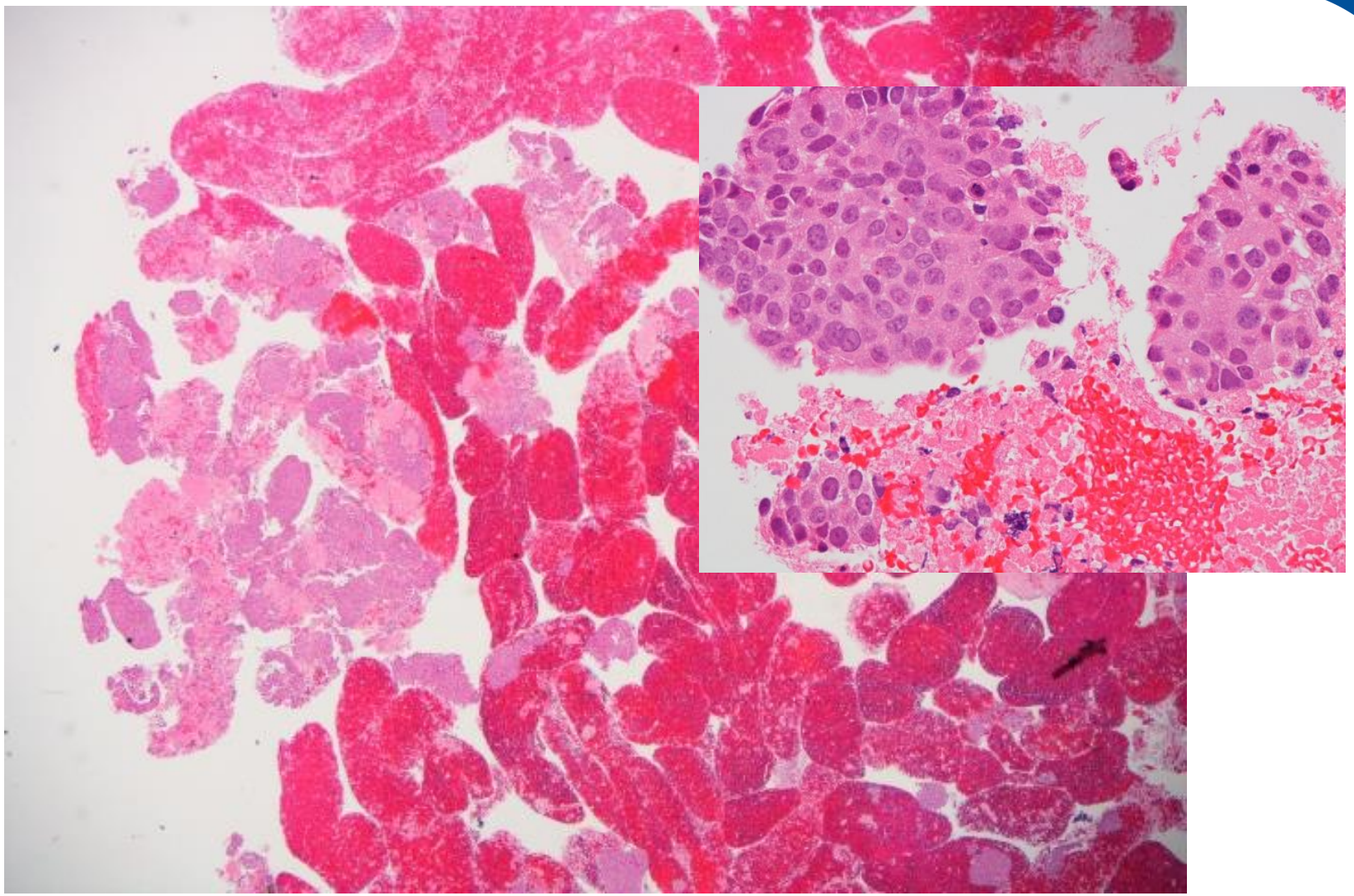
Pass 2

ROSE at West Herts



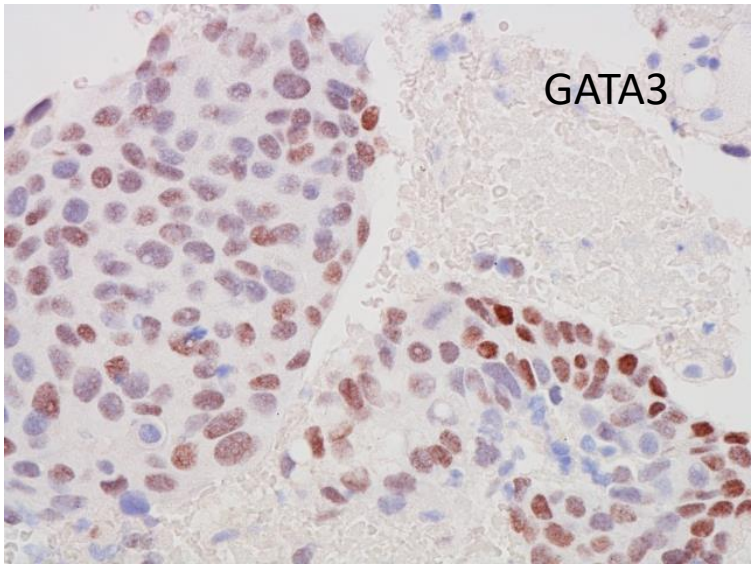
Pass 2

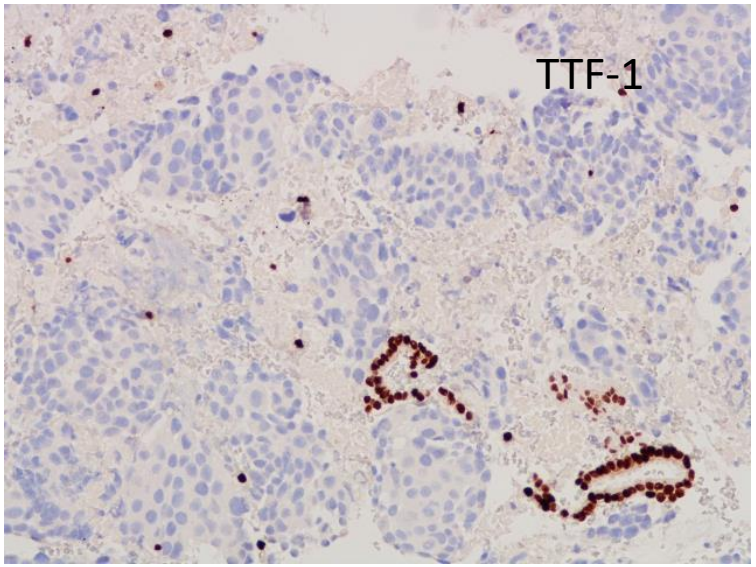
ROSE at West Herts

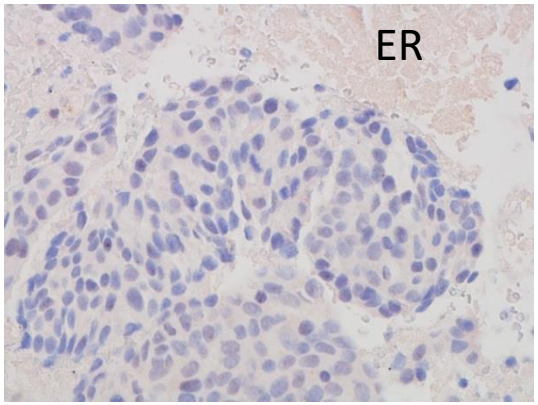


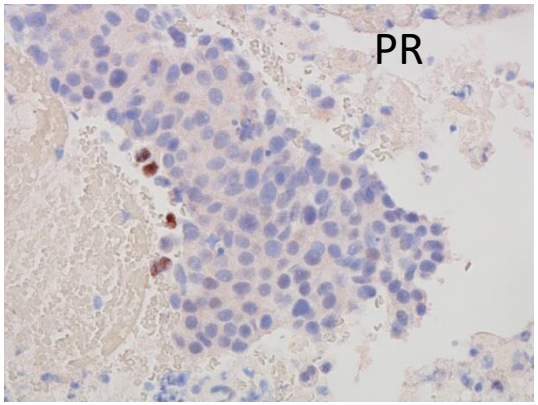
Cell block 1

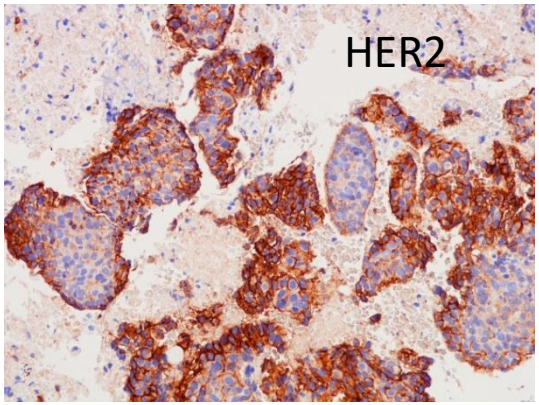
ROSE at West Herts





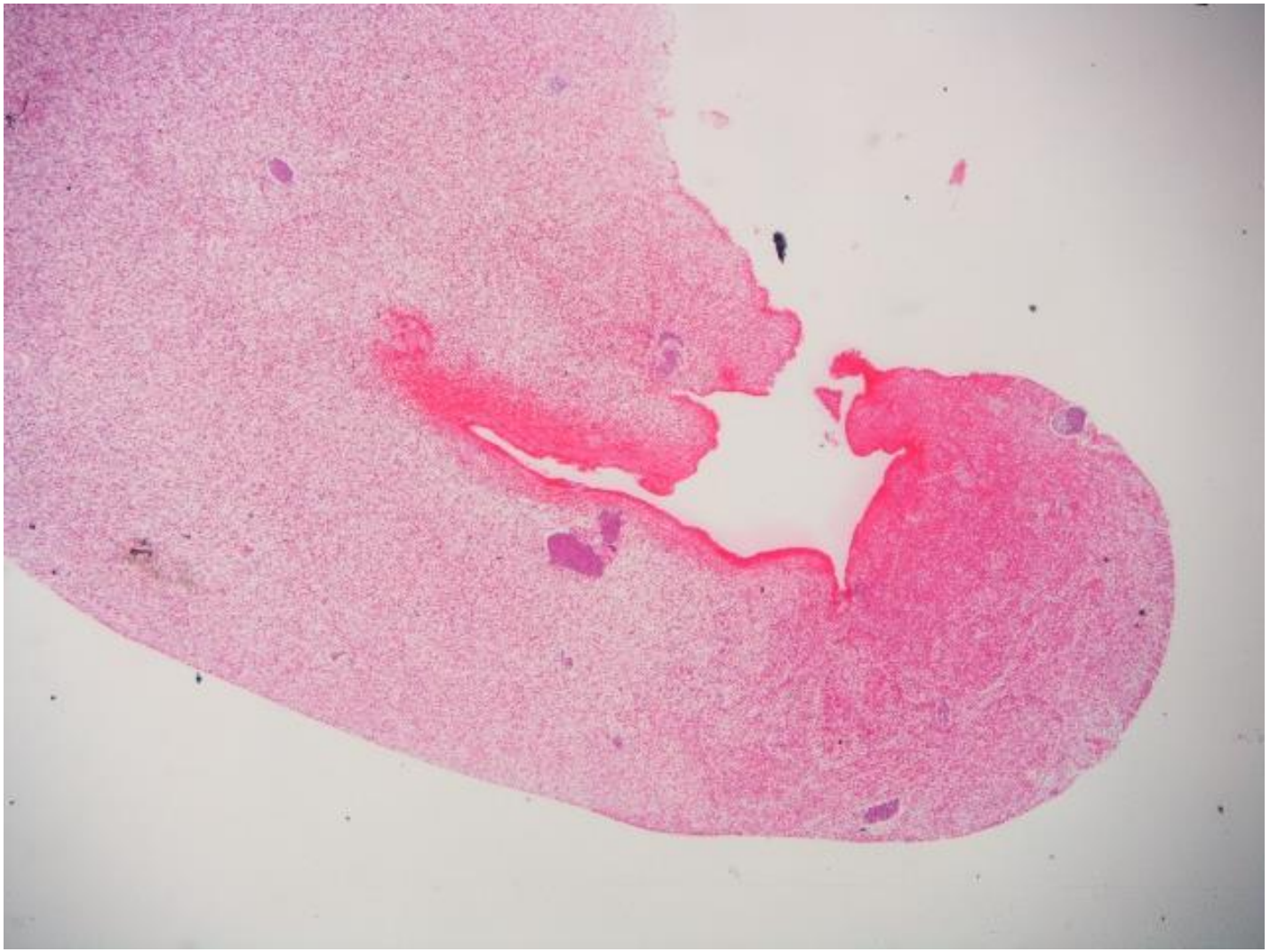






Cell block 1

ROSE at West Herts



Cell block 2

ROSE – who's in the team?

- At West Herts (and most places in UK where service available)
  - Cytopathologist
  - Biomedical scientist
- Around the world
  - May have purely cytotechnologist (BMS) teams
  - Aarhus University Hospital – kappa coefficient for diagnosis 0.99 (Schacht et al. Cytopathology 2016;27(5):344-350)

ROSE – availability

- 2014
- Telephone survey of 147 respiratory MDTs
- 73 currently using EBUS
- 15 have ROSE
- (11 using EUS in addition to EBUS)
- Most MDTs unaware of ROSE as a technique

ROSE – who could/should be the team?

- Cytopathologist
- Biomedical scientist/cytotechnologist
- Endoscopist
- Radiologist

- Combinations of the above

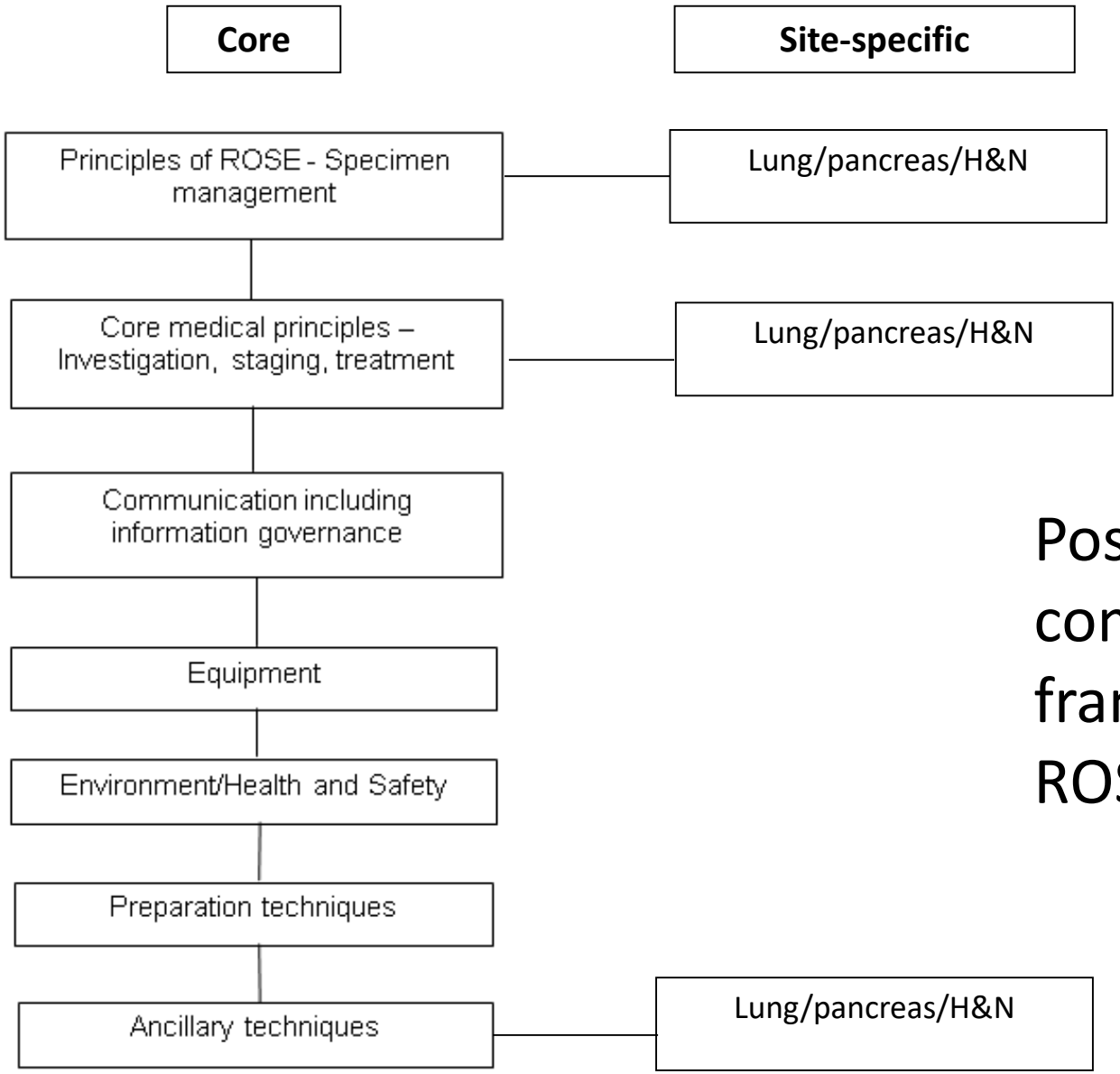
April 2016

BAC British Association for Cytopathology
IBMS Institute of Biomedical Science
The Royal College of Pathologists
Pathology: the science behind the cure

Role of Biomedical Scientists within the provision of a non-gynaecological cytology service

Sample assessment for adequacy for reporting

Certain NGC samples are taken by specific clinical procedures (e.g. mediastinal EBUS, FNA of many sites) by clinical teams or by Pathologists. An opinion as to sample adequacy and sometimes a diagnosis can be offered by a Pathologist at the time the sample is taken. In most settings though, resources do not allow for this. A comment on sample adequacy (Rapid on-site evaluation – ROSE) may be offered by a biomedical scientist. If the biomedical scientist has suitable experience based on competency and service needs and appropriate training/qualifications they may also be able to offer a preliminary opinion mainly for triage of the sample material rather than for patient management as well as ROSE.



Possible competency framework for ROSE

Summary

- The main benefit of ROSE is
  - Specimen management
  - Making the best use of valuable material
- Depending on site targeted and non-ROSE adequacy rate, may be beneficial for
  - Adequacy, diagnostic yield, efficiency of process
- In my view, best done by members of Cytology team, but not necessarily pathologists

Thanks to the West Herts ROSE team

- Winnie Tang, band 7 BMS and lead
- Claire Kiepura, band 6 BMS
- Claire Plank, band 6 BMS
- Maureen Grosso, cytoscreener
- Sharon Bunting, cytoscreener

Thank you